MOLECULAR APPROACHES TO THE STUDY OF THYROID HORMONE ACTION

Organizers: Jack Oppenheimer and Herbert Samuels

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Molecular Approaches to the Study of Thyroid Hormone Action

The C-erbA Superfamily of Genes: Biological Perspectives

L 001 MOLECULAR GENETICS OF THYROID HORMONE ACTION, R.M. Evans, C.Weinberger, C. Thompson, K. Damm, and K. Umesono, Gene Expression Lab, The Salk Institute, Howard Hughes Medical Institute, P.O. Box 85800, San Diego, CA

Five years ago the cDNA encoding the thyroid hormone receptor was cloned, and its resulting homology to the steroid receptors help to define the existence of the nuclear receptor superfamily. The TR is a hormone-dependent activator of transcription, yet even in the absence of ligand, the receptor can dimerize, bind to DNA and repress basal level transcription. Two distinct genes have been identified (alpha and beta) with multiple splicing variations. The v-erbA oncogene is a mutated derivative of the TR and provided the first evidence that nuclear receptors may be oncogenic. v-erbA cannot bind hormone, acts as a constitutive repressor and blocks the action of the wildtype TR. It is an example of a dominant negative oncogene.

Recently, we have characterized a transformation defective erbA mutant that harbors a single amino acid change in the carboxy terminus of the protein. Biochemical studies demonstrate that this mutant no longer functions as a transcription suppressor and is unable to function as a thyroid hormone receptor antagonist. Additional studies show that this amino acid is not critical for the DNA-binding, hormone binding or transcriptional activation properties of the wildtype receptor. This provides a direct association between transcriptional suppression and oncogenic transformation.

The receptor acts by binding to a set of target sequences known as thyroid hormone response elements or TREs. Two classes of TREs have been recognized, the inverted repeat and the tandem repeat. The inverted repeat closely resembles the estrogen response element and can also function as a retinoic acid receptor response element. Comparisons of the amino acid sequences in the DNA-binding domain of the thyroid hormone receptor to those of the steroid hormone receptors along with a detailed series of mutational studies has led to the identification of a discrete set of amino acids at the base of the first zinc finger, important in determining the primary sequence recognition (referred to as the "P" box). A second set of sequences in the amino terminal side of the second zinc finger has been shown to be important for recognizing the spacing of the half sites. This region may be important in receptor dimerization and has been referred to as the "D" box. The means by which the TR recognizes the tandem sequence motif is unclear, but seems likely to require an altered structural geometry. Sequences mediating this recognition pattern appear to be located in the carboxy terminus of the receptor and they include the conserved region known as the heptad repeat. Recent studies on the binding properties of the TR will be discussed.

L 002 PHYSICAL AND FUNCTIONAL INTERACTIONS AMONG THE THYROID/STEROID HORMONE RECEPTOR GENE FAMILY, Samuels, H.H., Forman, B.M., Au, M., Casanova, J., Ghysdael, J., Selmi, S., and Yang C.-R. Departments of Medicine and Pharmacology, NYU School of Medicine, NY, NY 10016 and Institut Curie, Section de Biologie, Centre Universitaire, Orsay, France (J.G.).

The thyroid (T3R) and retinoic acid (RAR) receptors are members of a subfamily of the steroid hormone receptor gene family which share several structural features. First, although these receptors can recognize different response elements, certain response elements are activated by both receptors. The native response elements recognized by these receptors consist of inverted repeats with no or minimal nucleotide gaps between half-sites or direct repeats with gaps of 4 to 5 nucleotides. The ligand binding domains of T3R and RAR contain a series of 8-9 hydrophobic heptad repeats which appear to play a role in homo- and hetero-dimer formation depending on the gap separation between half-sites. A DNA binding domain deficient (DBD$^-$) mutant of T3R functions as a dominant negative by inhibiting the activity of wild-type T3R and RAR. This activity of the DBD$^-$ mutant maps to the heptad repeat region suggesting that this dominant negative activity results from the formation of non-productive dimers with wild-type receptors. To study this in more detail we have expressed the chick T3R-alpha and the human RAR-alpha in E. coli and the DBD$^-$ T3R in E. coli and in a baculovirus expression system. The elements studied were an inverted repeat (IR) of an optimized TRE/RRE (AGGTCA TGACCT) with no gap between half-sites and with gaps of 3,5, and 10 nucleotides and a direct repeat (DR) (AGGTCA AGGTCA) and a DR with a 5 nucleotide gap (DR+5). T3R and RAR each bind to a half-site (AGGTCA) as monomers and to the IR as monomers and dimers. Formation of homo-dimers of RAR occurs with IR, IR+3, IR+5, and DR+5. T3R efficiently forms homo-dimers (vs. independent binding to two half-sites) only with IR and DR+5. The dominant negative activity of DBD$^-$ T3R is explained by inhibition of dimer formation by the mutant. Interestingly a DBD$^-$ v-erbA showed no dominant negative activity. This was found to result from one of the 9 amino acid changes in the v-erbA C-terminal region. Introduction of this mutation into DBD$^-$ T3R resulted in loss of dominant negative activity and this mutant was inactive in inhibiting T3R dimer formation. Formation of T3R/RAR hetero-dimers only occurs on elements which are dimer permissive for both receptors (e.g., IR and DR+5). In HeLa cells T3R interacts with a factor other than RAR to form a hetero-dimer only on elements which are dimer permissive for T3R. These studies indicate that T3R and RAR bind to half-sites and depending on the orientation and gap distance between half-sites interact to form homo-dimers or interact with other factors to form hetero-dimers. These results suggest that the diverse sequence organization of TREs and RREs promote a diversity of receptor-protein interactions which leads to selective changes in transcriptional activation of specific genes.

L 003 THYROID HORMONE RECEPTORS AND V-ERBA IN DIFFERENTIATION AND DEVELOPMENT,
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The v-erbA oncogene, a viral homologue to thyroid hormone receptor a (TRa), exerts its effects in erythroblasts by blocking their differentiation and by enabling the cells to grow in a wide pH range. We show here that these effects are mediated by transcriptional repression of erythroid specific genes. The expression of the erythocyte anion transporter (band 3) and carbonic anhydrase (CAII) are repressed by both v-erbB and overexpressed TRa in the absence of thyroid hormones, whereas addition of ligand restores normal transcription only with TRa, since the viral protein does not bind hormone. The results suggest that the highly expressed v-erbA gene acts by suppressing transcription of gene(s) that are not normally regulated by thyroid hormones. Furthermore, our data show that the repression of band 3 expression correlates with the oncogenic effect of v-erbA.
TRs have been grouped as a and b types and they derive from distinct genes. In chickens only a T3 binding form of TRa has been detected, contrasting the situation in mammals which express both T3 binding and non-binding forms of the a receptor as a result of alternative splicing. The chicken TRb also differs from the mammalian counterparts in that only 14 amino acids preceed its DNA binding region in its major form. An alternate form of this receptor, TRb-2, was in addition found in avian early embryonic eye. It differs from TRb-1 only in the N-terminal region. Due to the differences in TR structures between birds and mammals we suggest that thyroid hormones regulate development and metabolic processes differently in these organisms.
We tested the hypothesis that TRa and b differ in developmental functions by comparing their expression during chicken embryonic development. TRa mRNA was expressed in all tissues from earliest stages (day 4), with little variation in levels. In contrast, b mRNA expression was found mainly in brain, lung and yolk sac, and increased at late stages of development, e. g. the levels in brain and lung increased 20-30 fold upon hatching and beginning of respiration, respectively. In situ hybridization experiments have revealed that TRa is expressed in neuroblasts at the onset of the development of the cerebellum, whereas TRb only appears in neuroblasts of the definitive internal molecular layer. The expression pattern of TRb correlates well with known effects of thyroid hormone during development, and we suggest that these effects of the hormone are mediated by TRb. Specific effects of TRa are still unknown.

The Identification and Characterization of Thyroid Hormone Receptors and Related Gene Products

L 004 THYROID HORMONE RECEPTORS BIND TO DNA AS MOMOMERS, DIMERS, AND IN ASSOCIATION WITH NUCLEAR PROTEINS,
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Thyroid hormone receptors (TRs) mediate the regulation of gene transcription by thyroid hormone (T3) by binding to T3-responsive elements (TREs) in target genes. This interaction signals changes in the local activity of RNA polymerase II, perhaps via nuclear "adaptor" proteins. We have been studying the binding of TRs to TREs in the absence and in the presence of nuclear extracts. TRs α1, β1, and β2, synthesized in (non-nucleated) reticulocyte lysates, form complexes with the palindromic TRE, AGGTCATGACCT, which can be visualized in a gel electrophoretic mobility shift assay. We have raised anti-TRα and anti-TRβ rabbit sera which specifically "supershift" the TRα1 and TRβ complexes, respectively. Unlike related transcription factors such as the estrogen receptor, which bind to their responsive elements almost exclusively as dimers, TRs interact with TREs as either monomers or dimers which can be distinguished by their gel electrophoretic mobilities and methylation interference patterns. Nuclei derived from T3-unresponsive (COS-7 kidney, JEG-3 choriocarcinoma) and T3-responsive (GH3 pituitary, liver) cells contain proteins which retard the electrophoretic mobility of TR:TRE complexes but not the TRE alone, suggesting that they bind directly to the TR. The proteins from different sources do not bind simultaneously, but rather compete for the same site located in the carboxyl terminal half of the TR. The protein-TRE complexes form at lower DNA concentrations in the presence of nuclear extract, indicating that the nuclear proteins increase either the formation or the stability of the complex. Work is in progress to determine if the nuclear proteins interact with TR monomers, dimers, or both. In summary, TRs form numerous complexes with TREs. Binding of TRs to TREs as monomers, dimers, and in association with other nuclear proteins could have important effects on T3-dependent transcriptional regulation.

L 005 THYROID HORMONE RECEPTOR GENE PRODUCTS AND THEIR TRANS-ACTIVATING FUNCTIONS IN A CELL CULTURE. Vera Nikodem, CEB, NIH, Bethesda, MD 20892.
The role of the two similar thyroid hormone receptor genes α and β is incompletely understood. We have shown that the 3' heterogeneity of the α receptor (TRα) mRNAs is the result of selection between two polyadenylation sites and subsequent processing, giving polypeptides with dissimilar carboxy termini and different ligand binding characteristics. Variant I (TRαVI or TRα$_2$) whose carboxy terminal 120 amino acids replaced the last 40 amino acids of the TRα does not bind thyroid hormones (T3, T4). The 2.6 Kb TRαVI mRNA was found in many tissues including those which lack high affinity T3 binding sites (testis) and is present in brain, where it exceeds the level of TRα by ≈6-fold. We have reported that the T3 response element (TRE) of the malic enzyme (ME) gene resides at -287 to -261 and is comprised of discrete non-contiguous regions to which the TRα but not TRαVI binds as a dimer. Recently, we identified a TRE in the 5' flanking region of the myelin basic protein (MBP) gene. Since the physiological role for the receptor subtypes is unclear we have examined transactivating functions of TRα, β and αVI on both ME and MBP TREs in the context of their promoters. The promoter of ME is GC rich, while that of MBP contains TATA- and CAAT-like sequences. In an NIH3T3 transient expression system either TRα or β, in the presence of T3, enhanced the expression of both ME352 and MBP256 CAT chimeras albeit to different levels. While TRβ was a slightly less potent transcription activator than TRα in ME352 CAT expression, it caused a dramatic increase of MBP256 CAT expression (>30-fold) when compared to TRα (≈4-fold). Cotransfection of TRαvI and TRβ (3:1) had a striking inhibitory effect on the TRβ mediated transcriptional activation of MBP256 CAT returning it nearly to a basal level. The hormonal responsiveness of MBP256 CAT expression in the presence of TRα, however, was not affected by TRαvI. Under the same experimental conditions the TRαvI did not inhibit substantially ME352 CAT-T3 expression induced by either receptor. However, when the ME TRE was placed upstream of a thymidine kinase promoter, an inhibitory effect of TRαVI on both α and β transactivation was evident, giving a greater effect on the T3 responsiveness elicited by TRβ than TRα. Our results suggest that there is receptor selectivity among T3 regulated genes depending not only on the TRE but also on those promoter sequences specific to a given T3 responsive gene. An additional gene specific effect is exerted by the variable inhibitory effectiveness of TRαVI.

Receptor-Receptor Interaction

L 006 INTERACTIONS OF WILD TYPE AND MUTANT THYROID HORMONE RECEPTORS WITH A NUCLEAR PROTEIN, Ronald J. Koenig, Amy L. O'Donnell, Evan D. Rosen, and Douglas S. Darling, Division of Endocrinology, University of Michigan Medical Center, Ann Arbor, MI 48109
A 20 amino acid region within the ligand binding domain of T3R-ß1 (aa 286-305) is highly conserved across the erbA superfamily. This conservation extends not only amongst the traditional steroid receptors, but also includes other members of this gene family such as Rev-ErbA, PPAR, COUP-TF, and H2RII-BP. Typically, these proteins share 65-85% sequence similarity within this segment. Although this region lies within the ligand binding domain of the T3R, the fact that erbA superfamily proteins bind structurally unrelated ligands (or possibly no ligands in some cases) would suggest a function for this domain distinct from ligand binding. We previously assessed the consequences of mutation within T3R-ß1 aa 286-305 (1). In those experiments, several mutations were identified that severely impaired the ability of the T3R to induce reporter gene expression from a T3 responsive construct in transiently transfected cells. However, these mutations did not affect ligand binding, nuclear localization, or binding to a T3 response element (TRE) in vitro. We now report further studies with these mutant T3Rs. Binding of wild type T3R-ß1 to either the rat growth hormone gene TRE or a palindromic derivative was enhanced up to 5 fold by a nuclear extract. However, T3R-ß1 with mutations in aa 286-305 showed little to no enhancement of DNA binding in the presence of nuclear extract. Thus, although DNA binding of the mutants was equivalent to that of wild type T3R in the absence of extract, the mutants bound DNA less well in the presence of extract. Extracts from many organs, including those that are classically unresponsive to T3 (spleen, testis, brain), contained this activity, which was trypsin sensitive. Chemical cross linking revealed the presence of a 63 kDa protein that interacts with wild type T3R-ß1, but not with aa 286-305 mutants. This protein is designated TRAP, T3 Receptor Auxiliary Protein. Studies also were conducted with T3R-α1, which showed a 4-5 fold increase in DNA binding in the presence of TRAP. Surprisingly, TRAP did not enhance DNA binding of either v-erbA or erbA-α2. However, nuclear extracts did enhance binding of retinoic acid receptor ß to the TRE/RARE. Conclusions: 1) a 63 kDa protein (TRAP) interacts with wild type T3R-α1 and ß1, but not with T3R-ß1 containing mutations in aa 286-305; 2) this TRAP-T3R interaction correlates with enhancement of DNA binding in vitro; 3) v-erbA, erbA-α2, and T3R-ß1 with mutations in aa 286-305 fail to exhibit enhanced DNA binding in the presence of TRAP and fail to function as transcriptional activators; 4) interaction with TRAP cannot be predicted simply by conservation of the 20 aa sequence T3R-ß1 aa 286-305, since this sequence domain is identical in T3R-α1 and erbA-α2.

1. O'Donnell AL, Koenig RJ 1990 Mol Endocrinol 4:715-720.

L 007 MECHANISMS OF TRANSCRIPTIONAL REPRESSION BY THYROID HORMONE RECEPTOR ISOFORMS, Magnus Pfahl, Cancer Research Center, La Jolla Cancer Research Foundation, La Jolla, CA 92037
Gene regulation by thyroid hormones is mediated through multiple nuclear receptors. The thyroid hormone receptor (TR) isoforms TRα1 and TRβ have been shown to function as transcriptional activators in the presence of the thyroid hormone T_3. In the absence of T_3, TRα1 and TRβ have also been shown to function as thyroid hormone response element (TRE) specific repressor/silencer proteins. Our data show that TRα1 and TRβ bind TREs efficiently in the absence of T_3, and form more stable complexes with the TRE than retinoic acid (RAR) and estrogen receptor (ER) that are also efficient TRE activators. This tight interaction of the TRs with the TRE is controlled by the ligand binding domain, and in the case of TRβ is reduced in the presence of ligand. The isoform TRα2 is not a transcriptional activator, but has repressor activity when co-transfected with TRα1, TRβ, RAR or ER. We have now shown that TRα2 functions as a repressor by a different mechanism different from TRα1. TRα2 appears to be unable to form homodimers and therefore binds only very weakly to the TRE. Our data support a mechanism in which TRa2 inhibits other receptors by heterodimer formation. Repression of receptor activity by protein-protein interaction may be a widely used mechanism that we and others have recently shown to also include other transcription factors, such as Jun and Fos.

L 008 DIMERIC AND HETERODIMERIC CONFIGURATIONS OF T_3 AND RETINOIC ACID RECEPTORS IN ACTIVATION OF GENE TRANSCRIPTION M.G. Rosenfeld, O. Devary, C.G. Glass, J. Holloway, S. Li, & V. Yu. Howard Hughes Medical Institute, University of California, San Diego, School of Medicine, 9500 Gilman Drive, La Jolla, CA 92093-0648. To investigate mechanisms responsible for positive and negative transcriptional control, we have utilized two types of promoters that are differentially regulated by thyroid hormone (T_3) receptors. Promoters containing the palindromic T_3 response element TCAGGTCA TGACCTGA are positively regulated by the thyroid hormone receptor after the administration of T_3, while otherwise identical promoters containing the estrogen response element TCAGGTCA <u>CTG</u> TGACCTGA can be regulated negatively; converse effects are observed with the estrogen receptor. We describe evidence that the transcriptional inhibitory effects of the T_3 and estrogen receptors on the estrogen or T_3 response elements, respectively, are imposed by amino acid sequences in the C'-terminal region that co-localize with dimerization and hormone binding domains, and that these sequences can transfer inhibitory functions to other classes of transcription factors. Further, several lines of evidence are presented for the existence of multiple, cell-type specific nuclear proteins, in addition to the β-T_3 receptor, that function to differentially increase the binding affinity of the alpha retinoic acid receptor for a variety of response elements. These proteins, which we refer to as retinoic acid receptor co-regulators (RAR-COR), interact with the retinoic acid receptor via a common dimerization interface that overlaps with its ligand binding domain. These observations raise the intriguing possibility that co-regulatory proteins may serve to restrict and/or direct the effects of retinoic acid receptors on patterns of gene expression during development.

Molecular Approaches to the Study of Thyroid Hormone Action

Interaction of Receptor with the Thyroid Hormone Response Element

L 009 TRANSCRIPTIONAL ACTIVATION AND REPRESSION BY THYROID HORMONE RECEPTORS. J. Larry Jameson, Takashi Nagaya, Laird D. Madison, V. Krishna K. Chatterjee, and Shoumen Datta, Thyroid Unit, Massachusetts General Hospital, Boston, MA 02114

Thyroid hormone (T3) activated receptors stimulate the transcription of some genes (e.g., rGH, malic enzyme, MHCα), but repress other genes (e.g., TSH α and ß). We used transient expression assays to study mechanisms of transcriptional activation (pTreTKCAT) and repression (TSHαCAT) by T3 receptors. Both transactivation and transrepression required receptor activation by T3 and occurred equally with either the α or ß forms of the receptor, but not with the α2 isoform. However, pathways of transactivation and transrepression were distinguished by cotransfection of wild type receptors with mutant ß receptors. Several different ligand binding domain (LBD) mutants of the ß receptor effectively inhibited both transactivation and transrepression, exhibiting a property referred to as dominant negative inhibition of cotransfected wild type receptor. To further define the contributions of the DNA binding domain (DBD) and the ligand binding domain (LBD) to this process, a chimeric construct (Gal4-ß) consisting of the Gal4 DNA binding domain (DBD 1-147) was linked to the ligand binding domain (LBD 169-456) of the ß receptor. Gal4-ß activated transcription of reporter genes containing the Gal4 recognition sequence (UAS) in a T3-dependent manner, confirming its activity as a chimeric transcription factor. However, Gal4-ß did not activate TRETKCAT, and it did not repress TSHαCAT, consistent with a requirement for the T3 receptor DBD for both forms of regulation. When Gal4-ß was co-transfected with wild type ß receptor, it had no effect on transrepression and it weakly inhibited transactivation. However, a LBD mutation in the Gal4-ß chimera that corresponds to the deletion (Δ448-456) in v-erbA strongly inhibited wild-type receptor transactivation, but had no effect on repression. These and additional data suggest that: 1) An intact DBD is required for both transactivation and transrepression; 2) Dominant negative inhibition by functionally inactive receptors likely occurs by several mechanisms including competition for receptor binding to DNA, formation of inactive heterodimers, and depletion of limiting cellular transcription factors; 3) Competition with hybrid DBD/LBD mutants distinguishes pathways for transactivation and transrepression.

L 010 DEFINING THE THYROID HORMONE RESPONSE ELEMENT, P. Reed Larsen, Gregory A. Brent and David D. Moore, Howard Hughes Medical Institute Laboratory and Thyroid Division, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School and Division of Molecular Biology, Massachusetts General Hospital and Department of Genetics, Harvard Medical School, Boston, MA 02115

Sequences of DNA necessary for thyroid hormone directed gene expression (T3 response elements or TREs) have been identified in the promoters of a number of thyroid hormone responsive genes. Positive regulatory function has been localized to specific sequences for the rat growth hormone, the α myosin heavy chain and the malic enzyme promoters. Sequences conferring negative regulation (nTRE) have been identified in rat and/or human α and β TSH subunit genes. There is a reasonably well conserved consensus, the hexamer AGGTC/AA which is represented with varying fidelity in all of these sequences. The GGT triplet is the most critical for function. A positive function response requires the presence of at least 2 copies of this hexamer half-site which can be arranged as either palindromic or direct repeats. While two such sites are the minimum required for a positive TRE, multiple copies may be present each of which contributes to the functional response. For example, recent gel shift studies with *in vitro* expressed T3 receptor have demonstrated that point mutations in any of the three half-site domains of the TRE of the rGH promoter alter receptor-DNA interactions. This would be predicted from the functional effects of these mutations. In addition to the sequences in each half-site, the position of the TRE relative to the transcriptional start site also influences the magnitude of the response. Thus a TRE-palindrome causes greater induction of the rGH promoter at -137 than at -55 or -900 though it can still confer a weak positive response at +14. In contrast, a negative TRE will confer a negative T3 response to the same promoter when placed at -55 and +14 indicating that the direction of the response is intrinsic to the TRE sequence. A remarkable aspect of the T3 response elements is that despite the basic similarities in the half-sites, there is considerable microheterogenity between them. The physiological implications of these differences remain to be elucidated.

L 011 THE ALTERNATE STIMULATION OR SUPPRESSION OF PROLACTIN GENE TRANSCRIPTION BY L-TRIIODO THYRONINE IS MEDIATED BY MULTIPLE RECEPTOR BINDING MOTIFS WITHIN THE PROXIMAL PROLACTIN GENE. Francesc Villarroya, Craig M. Rosen, and Frederick M. Stanley, Department of Medicine and Pharmacology, NYU Medical School, New York, NY 10016.

L-Triiodothyronine (L-T3) stimulates transcription of the endogenous prolactin gene in GH_3 cells while it inhibits it in GH_1 cells. To investigate the mechanism of L-T3 regulation of the prolactin gene, we have examined the effects of L-T3 on a panel of chimeric plasmids consisting of 5'-flanking DNA from the prolactin gene ligated to the bacterial reporter gene, chloramphenicol acetyl transferase (CAT). As with the endogenous gene, L-T3 stimulates Prl-CAT expression in GH4 cells while it inhibits it in GH1 cells. The L-T3 response element (TRE) was shown to be in the region of the prolactin gene from -176 to +75. We now present both functional and physical evidence that multiple L-T3-receptor binding motifs in the proximal prolactin promoter mediate these effects. Functional studies used various 5' and 3' deletions of the Prl-CAT (-46/+75, -35/+75, -173/+56, -173/+36, and -173/-17). L-T3 Stimulates Prl-CAT expression in GH4 cells using all of these constructs except the -173/-17. Thus a stimulatory TRE is between -17 and +35. This is confirmed using a plasmid in which -11/+75 prolactin sequences were cloned 5' to the basal growth hormone promoter. This plasmid also showed stimulation by L-T3. Inhibition by L-T3 is mediated by a negative TRE between -35 and -17 since all of these plasmids are capable of mediating the inhibition of prolactin gene transcription in GH1 cells. Comparison of the sequence of the prolactin gene with a proposed palindromic TRE (AGGTCATGACCT) revealed several possible sites of interaction with the L-T3 receptor. These were tested by gel-mobility shift analysis using bacterially expressed L-T3-receptor. L-T3-receptor binds with very high affinity (compared to a palindromic TRE) to a region of the prolactin gene, +15/+34, having two direct repeats of this sequence. It also binds with lower affinity to -56/-37, and -36/-17 which each have one copy of this sequence. These data support an inhibitory effect of L-T3 mediated by displacement of TATA element (-28/-23) associated transcription factors by L-T3-receptor acting at a relatively weak TRE. Experiments with a plasmid containing prolactin DNA -173/-11 cloned 5' to the basal growth hormone promoter support this. Transcription of this plasmid, in which putative negative element is situated at a distance from the growth hormone promoter TATA element, is not inhibited or stimulated by L-T3. The stimulation of the prolactin gene in GH4 cells could depend on the association of the L-T3-receptor with another transcription factor (not present in sufficient quantities in GH1 cells). However, we have also found that Na-butyrate reverses the effect of L-T3 in GH1 cells. Since the L-T3-receptor is phosphorylated, post-synthetic modification of L-T3-receptor, mediated by butyrate or natural factors, might change the affinity of the receptor for its TREs and thus alter its effects.

L 012 **T_3 RECEPTOR INTERACTIONS IN THE REGULATION OF HEPATIC GENE EXPRESSION,** Howard C. Towle, Nathan D. Zilz, Hsiu-Ming Shih and Hsien-Ching Liu, Dept. of Biochemistry, University of Minnesota, Minneapolis, MN 55455

The expression of the S_{14} gene is rapidly and markedly induced in liver following administration of T_3 to the rat and, thus, has served as a useful model for studying the molecular action of thyroid hormone. We have recently used the COS cell cotransfection assay to explore the DNA sequences responsible for this control. Using either α or β-forms of T_3 receptor, thyroid hormone response elements (TREs) were localized to a region far upstream (-2950 to -2450) from the S_{14} transcription initiation site. This location was consistent with the presence of a T_3-sensitive DNase I hypersensitive region mapped by Jump (J. Biol. Chem. 264: 4698-4703 [1989]). Within this region, each of 3 contiguous DNA segments ranging in size from 110 to 227 bp possessed TRE activity (4.6- to 24.8-fold) when linked to a basal S_{14} promoter containing 290 bp of 5'-flanking sequence. Each TRE segment could bind to *in vitro* translated T_3 receptor in a gel shift assay. To compare the activity of these TRE sequences under a more physiologically relevant setting, each of the above constructs was tested in a cotransfection assay using primary rat hepatocytes. In hepatocytes, a chimeric construct with 3300 bp of 5'-flanking region of the S_{14} gene gave a marked response to T_3 addition to the media, again with α and β forms of the T_3 receptor yielding a similar response. Surprisingly, none of the 3 individual TRE segments defined in the COS cell cotransfection assay was active in primary hepatocytes, indicating additional sequence requirements. However, a construct containing all 3 fragments (-2952 to -2448) or the downstream 2 segments (-2790 to -2448) gave activity equal to the full-length construct. One possible explanation for the difference in response between COS cells and hepatocytes was that multiple TREs are necessary to activate the S_{14} promoter in the hepatocyte. To test this possibility, a construct was made containing 4 copies of a synthetic palindromic TRE upstream of the S_{14} promoter. Although this construct was highly T_3 responsive in COS cells, it was only minimally active in the primary hepatocytes. These data indicate that additional DNA sequences and trans-acting factors are necessary to form a functional TRE which is active with the S_{14} promoter in the context of the hepatocyte. (Supported by NIH grants DK 26919 and DK39997.)

V-erbA and Oncogenes

L 013 POSSIBLE MECHNANISMS OF V- AND C-*ERB* A ONCOGENE FUNCTION IN LEUKEMIC TRANSFORMATION;

.Martin Zenke, Christine Disela, Christian Schroeder and Hartmut Beug, Institute of Molecular Pathology, Dr. Bohrgasse 7, A 1030 Vienna, Austria.

The v-*erb*A oncogene, one of the two oncogenes of the avian erythroblastosis virus (AEV), efficiently blocks erythroid differentiation and suppresses transcription of erythrocyte-specific genes. The overexpressed thyroid hormone receptor c-*erb*A (TR) effectively modulates erythroid differentiation and erythrocyte-specific gene expression in a T3-dependent fashion, when introduced into erythroid cells *via* a retrovirus. In contrast, the endogenous thyroid hormone receptor, probably due to its low expression level, did only weakly affect erythroid differentiation.The analysis of a series of chimeric v-/c-*erb*A proteins suggests that the v-*erb*A oncoprotein has lost one type of thyroid hormone receptor function (to regulate erythrocyte gene transcription in response to T3) but constitutively displays another function, that of a repressor of transcription in the absence of T3.

In contrast to studies done with synthetic TR-response elements using the mammalian receptor, v-erbA was unable to function as a dominant-negative repressor in avian leukemic cells. V-*erb* A failed to block both the function of c-*erb* A on differentiation of erythroblasts and in transactivation studies using the carbonic anhydrase II promoter when both c-erbA and v-erbA were expressed in cells at comparable levels. The possible significance of the TR and/ or related receptors such as the retinoic acid receptor for erythroid differentiation and leukemogenesis will be discussed.

L 014 PHOSPHORYLATION OF THE CHICK c-erbA -ENCODED THYROID HORMONE RECEPTOR AND OF ITS Gag-v-erbA ONCOGENIC VERSION.

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The chick c-erbA -encoded T3 receptor is phosphorylated in cells at two distinct sites located in its amino-terminal domain. The first site (Ser 12) which is highly conserved among members of the type receptor family is phosphorylated by casein kinase II or a protein kinase with similar specificity. The second side (Ser 28/29) is specific of the chick c-erbA receptor and its phosphorylation in cells is enhanced following activation of either the PKA or PKC signal transduction pathways. Only this second phosphorylation site has been retained in the oncogenic gag-v-erbA protein of avian erythroblastosis virus. The study of the properties of unphosphorylated mutant versions of these proteins in their ability to alter the differentiation of erythroblasts transformed by temperature-sensitive oncogenic protein tyrosine kinase (ts-v-erbB or ts-v-sea) at non permissive temperature support the hypothesis that these phosphorylation events are important to erbA protein function.

L 015 V- AND C- ERB A ACTION IN YEAST AND IN VERTEBRATE CELLS,

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Recent attention has focused on v-erb A, a retroviral-derived copy of a thyroid hormone receptor (c-erb Aα) gene, as an example of a dominant negative receptor allele: an oncogene that may establish the neoplastic phenotype not by mimicking, but by interfering with the actions of its normal cellular progenitor. In vertebrate cells the v-erb A protein does not respond to thyroid hormone and functions as a constitutive transcriptional repressor. We have developed a plasmid system for studying the mechanisms of action of the v- and c-erb A proteins in the yeast S. cerevisiae. Unexpectedly, the v-erb A protein was found to be a hormone-regulated transcriptional activator in yeast. Our results indicate that the ability of v-erb A to act as an activator or a repressor, and to respond to hormone, is determined by the cellular context in which it is expressed. We suggest that interactions with other cellular factors may play important roles in regulation of the transcriptional properties of the v-and c-erb A proteins. Several possible candidates for these regulatory factors will be discussed.

We have also explored the DNA binding properties of the v-erb A protein by biochemical and genetic dissection. Our results indicate that the ability of v-erb A to act in the neoplastic cell is distinct from the ability of the viral allele to suppress c-erb A action in transient transfections. It is possible that changes in the DNA binding properties of the v-erb A protein relative to those of the c-erb A progenitor are important for v-erb A function, and that v-erb A acts in the cancer cell not by interfering with thyroid hormone receptors, but rather by participating (positively or negatively) in some other hormone response network.

L 016 ROLE OF C-ERBA AND ITS ONCOGENIC FORM V-ERBA IN CELL DIFFERENTIATION AND MALIGNANT TRANSFORMATION,

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The v-erbA oncogene is a mutated form of the c-erbAα protooncogene which encodes thyroid hormone (T3) receptors. It was identified in the genome of Avian Erythroblastosis Virus (AEV). The v-erbA oncoprotein blocks the differentiation of chicken erythrocytic progenitors and activates the growth of fibroblasts and thereby contributes to malignant erythroleukemic and sarcomatogenic transformation.

We show that c-erbAα is responsible for the commitment to final differentiation of early erythrocyte progenitor cells by both activating the expression of differenciation genes like the carbonic anhydrase II gene, and repressing the proliferation. Expression of v-erbA in these cells abrogates the effects of c-erbA. Data on the interaction of c-erbA and v-erbA proteins on the promoter of carbonic anhydrase II gene will be presented.

In fibroblasts, we show that v-erbA abrogates growth inhibition mediated by retinoic acid receptors. Putative target cellular genes of the v-erbA oncoprotein in these cells are under investigation.

In order to understand how c-erbAα can be activated into an oncogene we have constructed several recombinants between v-erbA and c-erbA. These chimeric genes are beeing tested for their oncogenicity in fibroblasts and erythrocytic progenitor cells.

The Regulation of TSH, TRH and Growth Hormone

L 017 POSITIVE AND NEGATIVE, AND LIGAND DEPENDENT AND INDEPENDENT ACTIONS OF THYROID HORMONE RECEPTORS: INFLUENCE OF T_3 AND DNA STRUCTURE, John D. Baxter, Ralff C.J. Ribeiro, Fred Schaufele, Thomas N. Lavin and Peter J. Kushner, Metabolic Research Unit, University of California, San Francisco, CA 94143

Thyroid hormone receptors (T_3R's) can have positive or negative effects on gene expression, and have complex interrelationships with other transcription factors. We have examined the roles of DNA structure and T_3 in these processes. Unliganded human ß-T_3R's expressed from transfected vectors in U937 cells have no effect on a palindromic thyroid response element (TRE-pal) upstream from a mouse tumor virus promoter, but stimulate a TRE in the rat growth hormone (rGH) promoter. Addition of T_3 stimulates the TRE-pal by about 15-fold and the rGH-TRE by an additional 2-fold. Unliganded hß-T_3R's expressed in HeLa cells have no effect on basal transcription from a frog vitellogenin (fvit) TRE placed upstream from a thymidine kinase (TK) promoter, but inhibit the basal transcription from a TRE-pal. T_3 stimulates both TREs, and it blocks the estrogen receptor enhanced transcription from the fvit TRE. The cell-free synthesized hß-T_3R binds to the fvit DNA as both monomers and dimers, to a non-palindrome in chicken embryonic myosin (CEM) DNA as a monomer, and to a CEM DNA mutated to a palindrome as a dimer. The inclusion of a rat liver factor that enhances T_3R binding to various TREs abolishes the dimer binding due apparently to the formation of a heterodimer. These results show that both unliganded and liganded T_3R's can act as positive or negative regulators of transcription, bind to DNA as monomers, dimers and heterodimers and that both T_3 and the nature of the T_3R DNA binding site influence the direction and magnitude of the response and the nature of the T_3R-DNA interaction.

L 018 A GENETIC ANALYSIS OF THYROID HORMONE RECEPTOR DIMERIZATION Jae W. Lee and David D. Moore, Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114

The bacteriophage λ repressor protein consists of an amino-terminal DNA binding domain and a carboxy-terminal dimerization domain, both of which are essential for function. This modular architecture is quite reminiscent of the independent DNA and ligand/dimerization domains of the members of the nuclear hormone receptor superfamily. We have constructed a series of repressor-receptor hybrid proteins and expressed them in E. coli. Bacteria expressing a chimeric repressor in which bacteriophage dimerization domain is replaced by the ligand/dimerization domain from the TRβ1 receptor are fully resistant to λ infection. Thus, the dimerization function of this domain of the T3 receptor is fully active in E. coli. We are using the power of λ genetics both to analyse the details of the process of T3 receptor dimerization and to identify other proteins that can interact with T3 receptors.

Thyroid Hormone Action in the Heart

L 019 THYROID HORMONE EFFECTS ON SLOW/CARDIAC SR Ca^{2+} ATPase GENE EXPRESSION IN HEART AND SKELETAL MUSCLE CELLS, Wolfgang H. Dillmann, Daniel K. Rohrer, and Ronald Hartong, Dept. of Medicine, Univ. of California, San Diego, 225 Dickinson St. (H-811-C), San Diego, CA 92103.

The heart is a major target organ for thyroid hormone. *In vivo*, changes in thyroid state are associated with marked changes in cardiac contractility, including a delayed speed of diastolic relaxation in the hypothyroid heart and an opposite change in the hyperthyroid heart. These changes in the rate of diastolic relaxation result from alterations in the activity of the slow/cardiac Ca^{2+} ATPase of the sarcoplasmic reticulum (SR). T_3 regulates Ca^{2+} ATPase activity at least in part at the mRNA level: administration of T_3 to hypothyroid rats leads to a 4 fold increase in the level of hybridizable cardiac Ca^{2+} ATPase mRNA within 24 hrs. To obtain more insight into the mechanisms by which T_3 regulates the expression of this gene, we first studied the effect of T_3 on SR Ca^{2+} ATPase mRNA levels in myocytes in culture. We found that addition of T_3 (100 nM) to rat neonatal cardiocytes, cultured in the presence of thyroidectomized calf serum, induced Ca^{2+} ATPase mRNA levels 3 fold within 8 hrs. The EC50 of T_3 is approximately 30 pM under serum-free conditions. In subsequent studies, a series of CAT expression vectors was constructed, driven by various fragments of the 5' regulatory region of the Ca^{2+} ATPase gene. Transient transfection of these constructs into myocytes, together with an expression vector encoding the human T_3-β receptor, showed that sequences residing between nucleotides -268 and -328 are capable of conferring T_3-responsiveness to a reporter gene, and sequences located between -565 and -328 are required for maximal transactivation. Inspection of the sequence of the 565 5'-flanking nucleotides reveals two areas with strong homology to TREs found in the rGH and MHC α gene: region I (position -530 to -511) contains two closely spaced TRE half sites in direct repeat, and region II (position -332 to -296) contains four closely spaced half sites (one inverted and three direct repeats). Region II is preceded by a 7 bp sequence which bears strong homology to the M-CAT motif, important for skeletal muscle-specific expression of the fast troponin gene; furthermore, a perfect MyoD binding site is observed between the CCAAT and the TATA boxes. The presence of muscle-type specific cis-acting DNA sequence elements in the regulatory region of the Ca^{2+} ATPase gene prompted us to investigate the response of this gene to T_3 in various muscle types. In contrast to the heart, we found that T_3 decreases the level of Ca^{2+} ATPase mRNA by 50% in fast/twitch skeletal muscle; this effect of T_3 is mimicked in the L6 cell line. In summary, expression of the slow/cardiac SR Ca^{2+} ATPase gene is markedly thyroid hormone responsive in heart and skeletal muscle cells. It appears that specific factors are able to modify the T_3 responsiveness of this gene in a cell-type specific manner.

L 020 HEMODYNAMIC AND THYROID HORMONE REGULATION OF MYOSIN HEAVY CHAIN GENE EXPRESSION IN THE HEART. Irwin Klein, North Shore University Hospital/Cornell University Medical College, Manhasset, NY 11030

Cardiovascular manifestions are some of the most prominent clinical findings associated with thyroid disease. In response to thyroid hormone (T_4) administration there is a prompt increase in cardiac size (hypertrophy) and a marked shift in the myosin isoform. The myosin heavy chain (MHC) filament consists of a dimeric combination of either alpha or beta MHC subunits which are products of separate genes. While it has been well established that T_4 can directly modulate MHC gene expression, the T_4-mediated changes in cardiac hemodynamics have also been identified as important regulators of cardiac growth and of the MHC isoforms. We have studied the rates of total protein and myosin synthesis, 18S RNA, total MHC mRNA, alpha and beta MHC mRNA content in the hemodynamically unloaded heterotopic cardiac isograft. Oligonucleotide probes (40 mer) specific for the non translated 3' end of the alpha and beta MHC genes were used to identify specific mRNA. In the isograft, as soon as 72 hours after transplantation in a euthyroid animal, myosin heavy chain protein synthesis declines by 50%, 18S RNA content declines by 16% but MHC mRNA was unaltered. In contrast, the alpha MHC mRNA content was decreased by 30% and there was de novo induction of beta MHC mRNA. Studies of protein synthetic efficiency suggests a preferential transcription and translation of beta MHC mRNA which occurs in euthyroid hearts undergoing atrophy. These findings, coupled with the observations that 1) T_4 does not promote protein synthesis in the iosgraft, 2) in vivo MHC gene expression correlates with heart rate rather than with T_4 levels and 3) that cardiac work stimulates beta MHC independant of the effects of T_4, further supports the notion that hemodynamics is a major determinant of cardiac growth and MHC expression in experimental thyroid disease.

L 021 MYOSIN HEAVY CHAIN GENES: A MODEL SYSTEM TO STUDY T_3-REGULATED GENE EXPRESSION. Vijak Mahdavi, W.Reid Thompson and Bernardo Nadal-Ginard, Department of Cardiology Children's Hospital and Department of Pediatrics, Harvard Medical School, Boston MA 02115.

The sarcomeric α- and β-myosin heavy chain (MHC) genes provide an advantageous model system to study the molecular mechanisms of thyroid hormones action. In rat, these genes are co-expressed in the ventricular myocardium but respond in an antithetic fashion to T_3, suggesting differences in their cis-acting regulatory sequences. In human, the β-MHC gene is less sensitive to T_3, suggesting cis- and/or trans-acting mechanisms for the interspecies differences of β-MHC gene expression.

T_3-mediated induction of rat and human α-MHC gene expression involves the interaction of a single T_3 responsive element (TRE) with T_3 receptors. The α-MHC TRE which consists of two direct repeats of the motif 5'GAGGT/$_A$C/$_G$ANN3' is identical in these two genes, and resides in their 5' upstream sequences, between -153 and -134. In one copy, this element confers up to 10-fold T_3-mediated induction of expression to heterologous promoters, in all cell types tested.

Expression of both human and rat β-MHC gene is mainly controlled by a cardiac and skeletal-muscle specific enhancer delineated between -354 and -180 upstream of the respective transcription start sites. The rat β-MHC enhancer, which is down-regulated by ~80% upon addition of T_3 and receptor, contains a single copy of the GAGGTCANN motif. This sequence binds to T_3 receptors with high affinity. Deletion of this element abolished the T_3 responsiveness of the rat β-MHC enhancer, whereas duplication of this negative TRE as a direct repeat or as a palindrome conferred T_3-dependent induction of transcription to homologous and heterologous promoters. In addition, the corresponding sequence in the human β-MHC enhancer: GTGGTCTNN, failed to bind T_3 receptors and did not confer T_3 responsiveness.

L 022 THYROID HORMONE ACTION IN THE HEART. E. Morkin, J.G. Edwards, R.W. Tsika, J.J. Bahl, S. Goldman* and I.L. Flink. University Heart Center, Arizona Health Sciences Center, Tucson, AZ 85724, and *Tucson Veterans Administration Hospital, Tucson, AZ 85723

Thyroid hormone is a powerful regulator of cardiac performance, increasing the strength and frequency of cardiac contraction by at least three separate physiological mechanisms that now can be identified by use of thyroid analogs. One of these mechanisms, which is thought to involve a switch in myosin heavy chain (MHC) isoforms, has been studied in detail. 3,5,3'-triiodo-L-thyronine (T_3) stimulates expression of the α-MHC gene and suppresses β-MHC mRNA production. These effects are mediated through nuclear thyroid hormone receptors (TRs), which are the products of the α and β c-erbA protooncogenes. T_3 response elements (TREs) and other positive and negative elements have been identified in the 5' flanking sequences of the human α- and β-MHC genes by transient expression of MHC/chloramphenicol acetyltransferase (CAT) fusion constructs in primary cultures of fetal rat cardiomyocytes. In addition, the interaction of MHC genes with TRs has been studied by cotransfection of MHC genes and expression vectors encoding $TR_{\alpha1}$, $TR_{\alpha2}$, and $TR_{\beta1}$ into receptor deficient CV-1 cells and fetal rat heart cells. In the human α-MHC gene, two TREs and a series of positive and negative regulatory elements were identified. The stronger-binding TRE contained two imperfect direct repeats with the consensus sequence 5'-C(T/A)GGAGG(T/A)-3'. In the human β-MHC gene, two pairs of TREs were tentatively identified located on each side of a strong positive element (SPE). A suppressor element also was present immediately 5' upstream to the SPE. One TRE in the proximal region was similar to a TRE found in α-TSH gene. Cotransfection with $TR_{\alpha1}$ enhanced T_3-induced activity of the α-MHC reporter plasmid over the level obtained with endogenous receptors both in cardiomyocytes and CV-1 cells. However, the reduction in activity of the β-MHC reporter plasmid by T_3 in cardiomyocytes was only slightly greater when cotransfected with TRs. The α_2 variant, which does not bind T_3, reduced induction of α-MHC fusion genes by T_3 in CV-1 cells, but had no effect in cardiomyocytes. The results provide an indication of the elements responsible for expression of the cardiac MHC genes and suggest that the actions of T_3 on the α- and β-MHC reporter genes may be mediated through either the $TR_{\alpha1}$ or $TR_{\beta1}$ receptor isoforms.

Inter-Relationship of Thyroid Hormone and Carbohydrate Metabolism

L 023 REGULATION OF TRANSCRIPTION OF THE MALIC ENZYME GENE, Alan G. Goodridge, Lisa M. Salati, Stephen A. Klautky and Cesar Roncero, Department of Biochemistry, University of Iowa, Iowa City, IA 52242. In chick-embryo hepatocytes incubated in a defined medium containing insulin, triiodothyronine (T3) stimulates about a 100-fold increase in malic enzyme (ME) activity. Insulin has little or no effect by itself. Glucagon or cAMP almost completely block the stimulation by T3. T3 caused a 30- to 40-fold increase in transcription of the ME gene; T3 for 1 h increased transcription to about 80% of maximum. Glucagon or cAMP for 1 h almost completely inhibited the response whether added simultaneously with T3 or after a 24 h incubation with T3. Inhibition of protein synthesis had no effect on the stimulation of transcription of the ME gene caused by 1 h with T3. These results suggest that transcription is the primary regulated step for both T3 and cAMP, and that interaction of T3 with its DNA-bound receptor directly regulates transcription. Similarly, cAMP may regulate the activity of a factor(s) that binds to the ME gene and controls its transcription. Chimeric genes containing 0.4 or 5.3 kb of 5'-flanking DNA of the ME gene ligated to the chloramphenicol acetyltransferase (CAT) gene were transfected into hepatocytes, with or without co-transfection of a T3 receptor gene. Using the 0.4 kb construct without over-expression of the T3 receptor, T3 and cAMP had virtually no effect on CAT activity; with a co-transfected T3 receptor gene, T3 stimulated a 30-fold increase in CAT activity, but cAMP had no effect. Using the 5.3 kb construct without an added T3 receptor gene, T3 caused a 15-fold increase, and cAMP a 70% decrease, in CAT activity; with a co-transfected T3 receptor gene, T3 stimulated CAT activity more than 100-fold, and cAMP had no effect. These and other results suggest the presence of at least two T3 regulatory elements in the malic enzyme gene. One is within 0.4 kb of the transcription start site and is weak; the other is between 0.4 and 5.3 kb upstream and is stronger. There is a cAMP regulatory element between 0.4 and 5.3 kb upstream; its action may be closely linked to that of the upstream T3 regulatory element.

T3, insulin and glucagon are not the only agents that regulate expression of malic enzyme in chick-embryo hepatocytes. The experiments just described were conducted during the first three days in culture. If the culture period is extended beyond three days, the response to T3 diminishes rapidly. Corticosterone preserves a substantial fraction of the responsiveness to T3. This effect of corticosterone is exerted at the transcriptional level. In addition, medium chain fatty acids inhibit T3-induced malic enzyme activity. Hexanoate probably acts directly on transcription initiation because near maximal inhibition is observed at 1 h after adding fatty acid to the medium. In summary, regulation of transcription of the malic enzyme gene is a complex process involving hormones and metabolites, as well as tissue-specific factors.
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L 024 THYROID HORMONE REGULATION OF THE S14 GENE IN LIVER AND 3T3-F442A ADIPOCYTES, Donald Jump, Gerald Lepar, Ormond MacDougald, Aneka Bell, Vivian Santiago and Darren Hu, Physiology Department, Michigan State University, East Lansing, MI 48824
The regulation of expression of the hepatic S14 protein is similar to that found for many lipogenic enzymes, including fatty acid synthase. T_3, insulin and dietary carbohydrate rapidly induce S14 gene transcription, while hormones which elevate hepatic cAMP levels suppress S14 gene transcription. To understand how T_3 interacts with other regulatory networks to regulate S14 gene transcription, we have taken two approaches to locate prospective cis-acting elements. An in vivo approach uses chromatin structure analysis, while an in vitro approach examines rat S14-CAT fusion gene expression in the 3T3-F442A adipocyte culture model. In the adult rat, the principal target for T_3 action corresponds to a DNase I hypersensitive site (HSS) located between 2.6 and 2.7 kb upstream from the transcription start site. T_3 induction of this HSS precedes T_3 mediated gene activation. Insulin-dependent dietary carbohydrate induction of S14 gene transcription is accompanied by the induction of two HSS. One HSS is located within the S14 proximal promoter and the other is located at -1.2 kb. Administration of T_3 or sucrose alone to starved hypothyroid rats induced S14 gene transcription to only 30% of the euthyroid level. However, administration of both T_3 and sucrose induced transcription to euthyroid levels within 4 hr. Thus, T_3 and insulin/dietary carbohydrate interact to regulate both S14 gene transcription and chromatin structure. S14 gene expression in 3T3-F442A adipocyte is dependent on expression of the adipocyte phenotype and the presence of glucocorticoids. Stable transfection analysis in adipocytes using S14-CAT fusion genes has located prospective glucocorticoid, insulin and tissue-specific cis-acting elements within -2.1 kb of the transcription start site. In contrast to in vivo studies, T_3 does not regulate S14 gene expression in adipocytes. This lack of T_3 control is due to suppressed expression of functional receptors (200-400 receptors/cell) and enhanced expression of a non-ligand binding variant, i.e. erbA-α_2. Surprisingly, retinoic acid regulates S14 gene transcription, but this action is dependent on the presence of glucocorticoids. Retinoic acid receptors do not utilize the S14 TRE or any RARE within 4.3 kb of the S14 transcription start site. Studies with the embryonic 3T3-F442A adipocytes suggest that retinoic acid may play a role in the ontogeny of adipocyte lipogenesis. Our transfection and chromatin structure analysis indicate that multiple cis-acting regulatory elements flank the 5'end of the S14 gene implicating a requirement for higher ordered chromatin folding to promote interaction between elements for hormone responsiveness.

L 025 DIETARY CARBOHYDRATE INCREASES TRANSCRIPTION, STABILIZES THE PRIMARY TRANSCRIPT, AND ENHANCES THE RATE OF PROCESSING OF THE mRNA-S14 GENE, Cary N. Mariash, Lynn A. Burmeister, and Yoshinori Goto, Divison of Endocrinology, Depts of Medicine and Cell Biology and Neuroanatomy, Univ of Minnesota, Minneapolis, MN 55455.
Hepatic mRNA-S14 rapidly responds to both thyroid hormone and dietary carbohydrate. To understand further the mechanisms of regulation of this mRNA by dietary factors, we used an in vivo kinetic analysis to analyze the regulation of nuclear processing. In addition, we also used transient transfection assays with the chloramphenicol acetyltransferase (CAT) reporter in different cell lines to study the factors that regulate transcription of this gene. In hypothyroid rats, changing from a regular chow diet to a lipogenic diet (fat-free, 60% sucrose, 20% casein) led to rapid accumulation of mRNA-S14 (2.2±0.6 chow fed vs. 13.5±2.5 pg/µg RNA lipogenic diet) by 4 hours. We used RNAse protection assay to measure the content of the primary transcript and found only a small increase in this transcript (0.14±0.01 chow fed vs 0.25±0.04 pg/µg RNA lipogenic diet). Also, the molar ratio of the mature:precursor mRNA-S14 content was lowest in the fasted state, (2.1), rose in the chow fed (16.5), and was the greatest on the lipogenic diet (62.7). This 30-fold increase in the mature:precursor ratio stands in contrast to the 3- to 5-fold increase in sucrose induced transcription previously observed. Stabilization of the mature mRNA-S14 cannot account for the lipogenic diet induced increase because the half-life of the mature mRNA is approximately 90 minutes in both the chow and lipogenic diet states. Therefore, the alteration in mature:precursor mRNA must be due to both an increase in the rate of processing and stabilization of the primary transcript. We also examined the transcriptional regulation of this gene in two cell lines, the fibroblast like CHO cell, and the glucose responsive Hamster pancreatic ß-cell (HIT). In the CHO cells the vector pS14•CAT(5Kb), containing 5Kb of 5'-flanking DNA, showed a 2-fold response when fructose was present and glucose was absent from the media. The fructose response required insulin and was absent when only 2Kb of 5'-flanking DNA was used. Metabolic studies in the CHO cell line showed that the cell did not use fructose, but used pyruvate for its energy source when glucose was absent. In the presence of glucose, pyruvate was not used. The response in HIT cells contrasts to that in CHO cells. An increase in the media glucose content from 2.2mM to 27.5mM led to a 4-fold increase in CAT activity with the vector pS14•CAT(5Kb). In addition, pS14•CAT(2Kb) also showed a glucose induced increase in CAT activity, but the response was less than that with pS14•CAT(5Kb). Overall, in HIT cells pS14•CAT(5Kb) gave a 15-fold greater response at 27.5mM glucose than pS14•CAT(2Kb) at 2.2mM glucose. Metabolic studies confirm HIT cells use glucose via the TCA cycle to oxidize pyruvate. Thus there are multiple carbohydrate response elements in this gene which enhance the transcription of mRNA-S14. The functional activity of these response elements requires pyruvate oxidation, and the fuel used for pyruvate oxidation is cell specific. Also, at least in the liver, carbohydrate feeding alters the processing of the primary transcript of mRNA-S14.

L 026 ACTIVITY OF NUCLEAR FACTORS THAT REGULATE S14 GENE TRANSCRIPTION IS INFLUENCED BY THYROID HORMONE, Norman C.W. Wong, Departments of Medicine and Medical Biochemistry, University of Calgary, Calgary, AB, CANADA, T2N 4N1.
Results of previous studies demonstrated the binding of at least two nuclear proteins to a region of S14 chromatin, HS-1 that is preferentially digested by DNase I. HS-1 is located immediately adjacent to the transcription initiation site and found only in tissues, such as the liver where the gene is abundantly expressed and regulated by T3. Therefore, the proteins associated with HS-1 are expected to play important roles in regulating expression of the gene. Using gel retardation and DNase I footprinting techniques we have identified two hepatonuclear proteins, PS-1 and P-1 that bind to -63 to -48 and -310 to -288 of S14 DNA, respectively. PS-1 binds to the putative CAAT-motif of the gene and binding activity correlated with levels of the mRNA. Results arising from the use of a cell-free *in vitro* transcription assay showed that increased S14 promoter activity correlated with binding activity of the protein in response to the thyroid state of the animal. Displacement of PS-1 binding from the template DNA decreased S14 promoter activity in reactions containing hepatonuclear extract from hyperthyroid but not hypothyroid animals. A 7-8 fold increase in transcription was observed using a template that contained three PS-1 binding sites inserted 5' to a S14 promoter with minimal activity. In contrast to the correlation between PS-1 binding activity and mRNA-S14, an inverse relationship was observed with P-1 DNA binding activity and S14 gene expression. P-1 DNA binding activity was maximum in neonatal rats but decreased progressively with age, whereas mRNA-S14 increased steadily with senescence. Another example of the inverse correlation was found in genetically obese Corpulent rats where high levels of hepatic mRNA-S14 was accompanied by decreased P-1 activity. The exact opposite was observed in lean littermates which had low levels of mRNA-S14 and increased P-1 activity. Since P-1 activity is absent in adipose tissue, no difference in the levels of mRNA-S14 was observed in epididymal fat from obese or lean animals. Together these findings suggested a repressor role for P-1. The repressor hypothesis was tested using cell-free in vitro transcription and transient transfection assays. Results from these studies were consistent with a repressor role for P-1 binding activity. In summary, we have identified two hepatonuclear proteins PS-1 and P-1 that bind to the HS-1 site of S14 DNA. The binding of PS-1 and P-1 to template DNA appeared to increase and repress, respectively activity of the S14 promoter *in vitro*.

Molecular Approaches to the Study of Thyroid Hormone Action

Thyroid Hormone and Thermogenesis

L 027 THYROID HORMONE REGULATION OF NA,K-ATPase SUBUNIT MESSENGER RNAS AND ENZYME ACTIVITY, Faramarz Ismail-Beigi, Department of Medicine, Columbia University, New York, NY 10032

Thyroid hormone (T_3) induction of target tissue Na,K-ATPase activity and abundance is preceded by increased abundances of the α- and ß-subunit mRNAs encoding the enzyme, and the latter effect is mediated, in part, by enhanced transcription of the corresponding genes. In both skeletal muscle and myocardium, tissues that express both the α1 and α2 isoforms of Na,K-ATPase, T_3 induction of $mRNA_{\alpha 2}$ and α2 enzyme far exceeds the stimulation of $mRNA_{\alpha 1}$ abundance and α1 activity. The 100- to 1000-fold higher sensitivity of the α2 isoform to inhibition by cardiac glycoside suggests an important physiological role for the preferential induction of α2 activity in hyperthyroid states.

In other studies the role of mRNA turnover in T_3-induced increase in subunit-mRNA abundances was explored in Clone 9 cells, a "non-transformed" rat liver cell line. Exposure of these cells to 10^{-8} M T_3 results in a stimulation of Na,K-ATPase activity and increased abundance of $mRNA_{\alpha 1}$ and $mRNA_{\beta 1}$ by 1.4- and 1.5-fold at 2 h, respectively (1). In the present studies, diluent- and T_3-treated cells were incubated in the presence of 150-200 μCi [^{3}H]uridine for 14-16 h, and the label was then "chased" by a change to fresh medium containing 5 mM uridine and cytidine and cytoplasmic RNA was isolated at 2, 6, 10, and 14 h thereafter. Total and [^{3}H]-labelled $mRNA_{\alpha 1}$ and $mRNA_{\beta 1}$ were quantified by Northern blotting and by specific hybridization to immobilized $cDNA_{\alpha 1}$ and $cDNA_{\beta 1}$, respectively. Measurement of the content of DNA, RNA, $mRNA_{\alpha 1}$, and $mRNA_{\beta 1}$ per plate of cells verified the presence of "steady-state" conditions during the 14-h "chase" period. In both diluent- and T_3-treated cells, the rate of decrease in [^{3}H]-$mRNA_{\alpha 1}$ and [^{3}H]-$mRNA_{\beta 1}$ content conformed to a single-exponential decay model with apparent half-lives of ~7.5 and ~17 h for the subunit-mRNAs, respectively. It is concluded that "stabilization" of these mRNAs is not an important mechanism in the rapid induction of $mRNA_{\alpha 1}$ and $mRNA_{\beta 1}$ by T_3.

1) Gick and Ismail-Beigi, Am. J. Physiol. 258: C544-C551, 1990.

L 028 TRANSCRIPTIONAL, POST-TRANSCRIPTIONAL, AND POST-TRANSLATIONAL REGULATION OF G-PROTEIN-LINKED RECEPTORS: MECHANISMS OF THYROID HORMONE ACTION,

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β-adrenergic receptors typify a populous group of cell-surface receptors that regulate adenylyl cyclase, cGMP phosphodiesterase, phospholipases A and C, as well as several ion channels via activation of G-proteins. Desensitization and down-regulation of these receptors is promoted by exposure of cells to agonists. Up-regulation is promoted by permissive hormones such as glucocorticoids, thyroid hormones and the morphogen, retinoic acid. Cross-regulation of receptors, providing a means for the integration of input signals among G-protein-linked pathways has been demonstrated recently. Mechanism(s) for the regulation of receptor function and expression have been proposed. Whereas short-term desensitization of receptor function appears to reflect a post-translational modification (*i.e.*, protein phosphorylation), agonist-induced down-regulation of receptor expression involves a post-transcriptional control (*i.e.*, altered stability of receptor mRNA). Analysis of S49 mouse lymphoma variants with mutations in the hormone-sensitive adenylyl cyclase pathway demonstrate that cyclic AMP and protein kinase A play pivotal roles in both desensitization and down-regulation. Permissive effects of hormones such as glucocorticoids include a prominent role for transcriptional control. Putative glucocorticoid-response-elements (GREs) obligate for steroid-induced up-regulation of receptor expression have been identified. Cross-regulation of G-protein-linked receptors is exemplified by short-term catecholamine-induced up-regulation of α_1-adrenergic receptor expression via β_2-adrenergic receptor stimulation. Agonist-induced down-regulation of α_1-adrenergic receptors follows in time and is mediated via solely an α_1-adrenergic mechanism. Thyroid hormones regulate the responsiveness of many target tissues to catecholamine actions mediated via β-adrenergic receptors. Heart, liver, and adipose tissue are three targets for permissive effects of thyroid hormones on β-adrenergic receptors. In heart and adipose tissue hypothyroidism blunts and thyroid hormone administration *in vivo* potentiates β-catecholamine action, whereas in liver the converse is true. Molecular explanations for the regulation of β-adrenergic receptor expression in liver, heart, and adipose tissue by thyroid hormones will be discussed.

L 029 FUNCTIONAL AND MOLECULAR INTERRELATIONSHIP OF THYROID HORMONE-INDUCED LIPOGENESIS, LIPOLYSIS AND THERMOGENESIS J.H. Oppenheimer, H.L. Schwartz, J.T. Lane, and M.P. Thompson, Division of Endocrinology and Metabolism, Department of Medicine, University of Minnesota, Minneapolis, MN 55455

In homeotherms, triiodothyronine (T3) increases total body lipogenesis, lipolysis, and thermogenesis. We have explored the functional interrelationships of these processes and have begun to map the metabolic circuits involved in their regulation by T3. Caloric expenditure in free-living male Sprague-Dawley rats was estimated from caloric balance measurements, a method which yielded values closely approximating those obtained by direct calorimetry. Daily intraperitoneal doses of 15 µg T3 /100 g bw/d caused an increase in caloric expenditure from a basal value of 26.5 ± 1.7(SEM) kcal/100 g/d to a new steady state of 38.1 ± 1.5 kcal/100 g/d. As gauged by measurements of total body oxygen consumption, caloric expenditure increased within 24 hrs. and was maximal by d. 4. However, food intake rose only after 4-6 d. of treatment and became maximal only by d. 8. Since total urinary nitrogen excretion did not rise within the first 6 d. and since there was no increase in hepatic phosphoenolpyruvate carboxykinase mRNA at any time, gluconeogenesis from protein sources did not supply the needed substrate during the early increase in calorigenesis. Total body fat stores fell approximately 50% by the 6th d. of treatment and accounted for the entire increase in caloric expenditure during the initial period of T3 treatment. Total body lipogenesis measured by tritium incorporation increased within one day and reached a plateau 4 to 5 d. after the start of T3 treatment. Although 15-19% of the increased caloric intake was channeled through lipogenesis, the metabolic cost of the increased lipogenesis accounted for only 3-4% of the T3-induced increase in calorigenesis. Our results indicate that fatty acids derived from adipose tissue are the immediate source of substrate for thyroid hormone-induced calorigenesis and that the early increase in lipogenesis serves simply to maintain fat stores. Since the mRNAs coding for lipogenic enzymes and the enzymes involved in cardiac contractility rise within 2-3 hours after T3 administration, long before oxygen consumption and lipolysis increase, our results suggest that T3 does not act by substrate depletion as a result of increased metabolism. Rather, T3 appears to induce in a coordinate fashion the mRNAs coding for the enzymes governing T3-regulated energy expenditure, lipogenesis, and lipolysis.

L 030 CELLULAR AND MOLECULAR BASES FOR A ROLE OF THYROID HORMONE IN THE CONTROL OF FACULTATIVE NON-SHIVERING THERMOGENESIS. J. Enrique Silva, Antonio C. Bianco, Stefan Rehnmark, Xiaoyang Sheng and J. David Kieffer, Department of Medicine, Thyroid Unit, Beth Israel Hospital and Harvard Medical School, Boston, MA 02215.- Thyroid hormone enhances *obligatory* thermogenesis (≈basal metabolic rate) by virtue of its stimulating action on innumerable metabolic pathways. Although *facultative* thermogenesis is known to be essential to cold adaptation, the protective effect of thyroid hormone from cold-induced hypothermia has been traditionally related to its effect on obligatory thermogenesis. Brown adipose tissue (BAT) is the main site of facultative non-shivering thermogenesis in small mammals including the human newborn. BAT thermogenesis is stimulated by the sympathetic nervous system (SNS). Before the finding of Type II T_4-5'deiodinase (5'D-II) in BAT, it was felt that thyroid hormone was merely permissive for the function of this tissue. We have shown that BAT 5'D-II is activated by the SNS and this activation generates large amounts of the active thyroid hormone, T_3, enough to nearly saturate BAT nuclear T_3 receptors (NTR). The key element in BAT thermogenesis is uncoupling protein (UCP), a 32,000 M_r mitochondrial protein which uncouples phosphorylation in the abundant BAT mitochondria. UCP allows a dramatic increase in BAT thermogenesis when the tissue is stimulated by the SNS. UCP synthesis in response to cold is blunted in hypothyroid rats. In keeping with the adrenergic activation of 5'D-II and its being the main source of BAT T_3, the response is readily restored by small doses of T_4; this effect of T_4 is prevented by blocking T_4 5'deiodination or 5'D-II activation. The normal UCP response to cold requires a high NTR occupancy, which explains why, in the absence of T_4, hyperthyroid levels of plasma T_3 are required to fully restore the response. The capacity of hypothyroid rats treated with T_4 or T_3 to stand cold (no hypothermia) correlates with UCP levels and not with liver α-glycerophosphate dehydrogenase or plasma T_3 levels, reflection of obligatory thermogenesis. Likewise, the thermogenic response (oxygen consumption) to cold correlates with BAT NTR occupancy rather than with plasma T_3. At a molecular level, UCP levels during these manipulations are parallel to those of UCP mRNA. Norepinephrine (NE), the SNS neurotransmitter in BAT, stimulates UCP gene transcription, but in the absence of T_3, the response is limited. T_3 amplifies the effect of NE by a factor of 4-5 but it does not stimulate UCP gene transcription in the absence of NE. Neither the effect of NE nor that of T_3 on UCP gene transcription requires on-going protein synthesis, suggesting that NE- and T_3-dependent signals directly interact at the gene level. In cold-exposed rats euthyroid rats the transcriptional response is brisk (7-8-fold) but transient (≤8 h). However, UCP mRNA levels stay high for up to 4 days, being sustained by a combination of more efficient processing of precursor UCP mRNA and stabilization of mature UCP mRNA. Preliminary evidence suggests that these two mechanisms are also T_3-dependent. Thus, thyroid hormone is essential for facultative thermogenesis. This adaptive function of thyroid hormone takes place in BAT, where locally generated T_3 interacts synergistically with the SNS to regulate the expression of UCP at transcriptional and post-transcriptional levels. (Supported by NIH grant DK42431)

Molecular Approaches to the Study of Thyroid Hormone Action

Thyroid Hormone in the Development of the Central Nervous System

L 031 INFLUENCE OF THYROID HORMONE ON BRAIN GENE EXPRESSION, Juan Bernal, Angeles Rodriguez-Peña, Britt Melstrom, Ana Perez-Castillo, Angel Santos, and Alberto Muñoz, Instituto de Investigaciones Biomédicas, Facultad de Medicina, UAM, Madrid, Spain.

Brain maturation is dependent on an adequate supply of thyroid hormone during critical periods of fetal and neonatal development. To define at the molecular biological level the effects of thyroid hormone on brain development we have performed two series of studies. On the one hand we have examined the developmental pattern of alfa and beta c-erbA mRNAs in the rat during the neonatal period by in situ hybridization . The mRNAs coding for alpha and beta c-ErbA show independent patterns of temporal and spacial expression. Alpha1, but not beta c-erbA mRNA can be detected in the brain as early as in embryonal day 14 and shows high levels of expression in neonatal brain in olfactory bulb, cerebral cortex, hippocampus and cerebellum. Beta1 c-erbA mRNA is expressed in the caudate at birth and later on increases in other areas such as cerebral cortex, hippocampus and amigdala. In other series of experiments and in order to identify brain genes under thyroid hormone control we have searched for cDNA clones of brain mRNAs whose expression might be altered in hypothyroid animals during the neonatal period. Neonatal hypothyroidism was induced by a combination of chemical and surgical thyroidectomy and resulted in near undetectable T4 and T3 levels in total brain or cerebral cortex. Clones were identified with probes prepared by subtractive or differential hybridization and those corresponding to mRNAs altered in hypothyroidism were further studied by Northern blot analysis. Results of screenings by subtractive hybridization indicated that there probably are not mRNAs expressed at the level of 0.05% or higher which are absolutely regulated by the thyroid status. Using RNA prepared from whole brains no effect of hypothyroidism was found on the expression of the astroglial genes, for example those coding for glial fibrillary acidic protein and glutamine synthetase. Among genes of neuronal expression, no alterations were found in the steady state levels of most mRNAs for example neuronal specific enolase, microtubule-associated protein 2, Tau, or nerve growth factor. In contrast a 2-3 times decrease was found in a novel brain-specific mRNA known as RC3. Levels of RC3 protein were also found to be lower by Western blot. The abundance of the mRNAs for the major myelin proteins PLP, MBP and MAG, expressed by oligodendrocytes, were also decreased in hypothyroid brains, and the effect of hypothyroidism depended on the brain region examined, with no effects in cerebellum and clear effects in cerebral cortex. Developmental studies on RC3 and MAG expression indicated that the corresponding mRNAs accumulate in the brain of normal rats during the first 15-20 days of neonatal life. A delayed accumulation occurred in hypothyroid brains and at reduced levels. Thyroid hormone treatment of hypothyroid animals restored partially the altered mRNA levels. These results demonstrate that thyroid hormone controls the steady state levels of particular mRNAs during brain development, although no information regarding the mechanism of thyroid hormone action is still available.

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L 032 IMMUNOFLUORESCENT LOCALIZATION OF THYROID HORMONE RECEPTOR PROTEIN β1 AND VARIANT α2 IN SELECTED TISSUES: CEREBELLAR PURKINJE CELLS AS A MODEL FOR B1 RECEPTOR MEDIATED DEVELOPMENT EFFECTS OF THYROID HORMONE IN BRAIN, Kevin A. Strait, Harold L. Schwartz, Virginia S. Seybold, Nicholas C. Ling and Jack H. Oppenheimer, Section of Endocrinology and Metabolism, Department of Medicine (K.A.S., H.L.S. and J.H.O.) and the Department of Cell Biology and Neuroanatomy (K.A.S., H.L.S., V.S.S. and J.H.O.) University of Minnesota, Minneapolis, MN. and the Whittier Institute, La Jolla CA. (N.C.L.).

Rat c-erbA β1 mRNA/mg DNA undergoes a forty-fold rise in cerebrum during the first 10 days of life, coincident with a rise in tissue triiodothyronine (T3) levels and T3-dependent brain development (1). In contrast, the other mRNA coding for a T3-binding protein (α1), undergoes only a 1.5-fold increase during this period. These data suggest that the β1 receptor may mediate the effects of T3 on brain development. However, c-erbA β1 mRNA/mg DNA in cerebellum is only 1/127th that in cerebrum. Since cerebellar development, especially dendritic arborization of Purkinje cells, is a T3 sensitive process, we assessed the levels of the β1 receptor protein in cerebellum during development. Antisera to unique peptide regions of β1 were raised. Their specificity was demonstrated by specific immunoprecipitation of the in vitro translated product; 85% immunoprecipitation of the T3 binding activity in hepatic nuclear extracts; and by Western blot analysis of hepatic extracts. Immunohistochemical studies utilizing anti-β1 antiserum stained liver nuclei but not testis nuclei which contain no T3 binding activity or β1 mRNA. In cerebellum, intense staining was observed in Purkinje cell nuclei with lesser staining of granule cells. Thus, there were abundant β1 receptor levels in cerebellum despite a low β1 mRNA content. Both the intensity of staining in Purkinje cell nuclei and immunoprecipitable β1 receptor binding capacity rose in the neonatal period. Antiserum to the non-T3 binding α2 variant protein was also prepared and a distinctive pattern of fluorescence observed. Strong fluorescence was seen in the nuclei of granule cells, but none in Purkinje cells. Alpha-2 protein content in the dividing spermatogonia in testis was high. Since testis contains no detectable T3 binding capacity, and no α1 or β1 mRNAs (1), the role of the α2 protein in the testis cannot be that of dominant negative regulation. Our findings support the concept that the β1 receptor plays a central role in T3-induced brain development and strongly suggest that the Purkinje cell is a direct target for T3.

(1) Strait, K. A., Schwartz, H. L., Perez-Castillo, A., and Oppenheimer, J. H. (1990) J. Biol. Chem. **265**, 10514-10521.

L 033 THYROID HORMONAL CONTROL OF MORPHOGENESIS AND CELL DEATH IN AMPHIBIAN METAMORPHOSIS. Jamshed R. Tata, Akira Kawahara, Elida Rabelo and Betty Baker, National Institute for Medical Research, Mill Hill, London NW7 1AA, UK.
Amphibian metamorphosis is a dramatic example of morphogenesis, cell death and genetic reprogramming, orchestrated by a single signal, in post-embryonic development. Thyroid hormones induce and regulate these diverse developmental changes directly in virtually every tissue of the tadpole. Earlier work had shown that *Xenopus* larval tissues acquire sensitivity to the exogenous hormone well before endogenous thyroid hormones are secreted, so that functional thyroid hormone receptors (TR) are expressed early in development. We now find that TR-α is the predominant, apparently unregulated, transcript at all stages before and during metamorphosis, and whose concentration declines after the froglet stage. The relatively low levels of TR-β gene expression, however, are developmentally regulated. *In situ* hybridization in early *Xenopus* tadpoles (stages 43 and 44) revealed high levels of expression of TR genes in the brain, a tissue known to be restructured during metamorphosis, and in the intestine and tail tip, tissues that are programmed for cell death. At prometamorphosis (stage 55), the hind limb bud, a tissue programmed for morphogenesis, also contained high levels of TR mRNAs. In order to better study the modulation of TR gene expression and the developmental action of thyroid hormones, we have succeeded in developing a system of organ culture of *Xenopus* tadpole limb buds and tails in which T_3 induces morphogenesis and cell death, respectively. Prolactin, a naturally occurring "juvenile" hormone in amphibia, abolishes the action of T_3 in these cultures. Data on TR gene expression in these two cultured tissues will be described. A comparison of the expression of TR genes during natural and T_3-induced metamorphosis with those encoding closely related c-*erb*-A type nuclear receptors, namely estrogen and retinoic acid receptors, will also be presented.

Thyroid Hormone Receptors in Man

L 034 MUTATIONS OF THE THYROID HORMONE RECEPTORS IN MAN RESULTING IN RESISTANCE TO THE HORMONE Samuel Refetoff, Kyoko Takeda, Akihiro Sakurai and Roy E. Weiss, Departments of Medicine and Pediatrics, The University of Chicago, Chicago Illinois, USA. Generalized resistance to thyroid hormone (GRTH) is a syndrome of reduced responsiveness of target tissues to thyroid hormone (TH). The clinical presentation is variable. At one end of the spectrum, affected subjects are euthyroid, their partial hormone resistance being compensated by elevated TH levels. At the other end, there is severe growth or mental retardation and in infants, typical cretinoid features. The syndrome is congenital and has been detected at birth by elevated blood thyroxine with non-suppressed thyrotropin, in the absence of TH transport defects. Inheritance is usually autosomal dominant, being recessive in only 5% of families. Soon after the description of the syndrome in 1967, it was postulated that the defect resides at the cellular level, most likely involving the putative thyroid hormone receptor (TR). Direct testing of this hypothesis became only recently feasible owing to the demonstration of genes that encode proteins which bind TH and function as TRs. The two genes, TRα and TRβ, located in human chromosomes 17 and 3, respectively generate each at least two isoforms, expressed in variable proportions in most tissues. A point mutation was first discovered in the TRβ gene of a subject with GRTH whose skin fibroblasts have been shown to be resistant to TH. Using fibroblast mRNA as template, cDNA was synthesized and the entire coding area of the TRβ was sequenced. A single G to C transversion was found in codon 345, replacing the normal Gly with Arg in one of the two alleles of the gene. His affected father had the same abnormality. In contrast to the wild type human (h) TRβ, the mutant TR (hTRβ-Mf) expressed in vitro was devoid of TH-binding activity. This was not surprising considering that the amino acid substitution is located in the TH-binding domain of the TR. The hTRβ-Mf bound normally to cis-acting TH response elements but had no activity when co-expressed with a reporter gene which functions in a TH-dependent manner. More importantly, hTRβ-Mf inhibited the transactivation regulated by the co-transfected normal hTRβ1 and hTRα1. Patients with GRTH from 24 unrelated families were screened by allele specific amplification for mutations identical to the hTRβ-Mf and for another mutant with reduced TH-binding activity, described by Usala et al. (Pro-453 $\rightarrow$ His). Mutations appear to be different for each family. In one family, affected individuals lacked the entire protein coding region of both hTRβ alleles. In this family with recessively inherited GRTH, the consanguineous,obligate heterozygous parents exhibited no clinical or laboratory abnormalities. Thus, manifestation of GRTH in heterozygous individuals requires the expression of a mutant receptor that interferes with the function of normal allele ("dominant negative effect"). Furthermore, complete absence of the hTRβ gene is compatible with life and expression of thyroid hormone action in such individuals is most likely mediated through the hTRα. The application of denaturing gradient gel electrophoresis on GC-clamped DNA fragments has facilitated the localization of mutations. Of the 22 families without major gene deletions, 18 were found to have point mutations in the hormone binding domain of the hTRβ gene. The 4 families with intact hTRβ most likely harbor mutations in the hTRα gene. Analysis by restriction fragment length polymorphism of two such families revealed no linkage to the hTRβ gene. Studying these errors of nature would help understanding the molecular mechanism of TH action.

L 035 A HOMOZYGOUS DELETION IN THE C-ERBAβ GENE IN A PATIENT WITH GENERALIZED THYROID HORMONE RESISTANCE (GTHR): CHARACTERIZATION OF THE MUTANT RECEPTOR S.J. Usala, B. B. Bercu, F.E. Wondisford, B.D. Weintraub. East Carolina University School of Medicine, Greenville, NC 27858; University of South Florida College of Medicine, Tampa, FL 33612; Case Western Reserve University, Cleveland, OH 44106; NIDDK, Bethesda, MD 20892
The gene for GTHR has been mapped to the c-erbAβ locus in multiple kindreds (1,2,3). Three point mutations have been reported in the T3-binding domain of this receptor gene: in Kindred A a C->A at nuc. position 1643 (2), in KIndred D a G->C at nuc. position 1305 (3), and in Kindred Mf a G->C at nuc. position 1318 (4). We recently reported that the Kindred A receptor had a diminished, but measurable, T3-binding affinity in contrast to the Mf receptor (5). We now report a novel genetic abnormality, a three base pair deletion in the T3-binding domain of c-erbAβ in Kindred S with GTHR. Heterozygotes from Kindred S harbored a CAC-deletion at nucs 1295-1297 which resulted in the deduced loss of amino acid residue threonine at codon 332. A patient, S1, from a consanguineous union in Kindred S, had two mutant alleles which resulted in markedly elevated TSH and free thyroxine levels and profound abnormalities in brain development and linear growth. A fibroblast c-erbAβ cDNA was cloned from patient S1 and used to create a full-length mutant cDNA. The Kindred S receptor synthesized in vitro did not bind T3. This mutant receptor did bind with similar avidity, when compared to wild-type β-receptor, to thyroid hormone response elements of the human TSHβ and rat GH genes. Kindred S showed the effect in man of heterozygous and homozygous expression of a dominant negative form of c-erbAβ. Further studies are in progress to study receptor dimerization in solution and cooperativity of T3-binding, and the effect of β-mutations.

1)Usala et al. Mol. Endocrinol. 2: 1217 (1988) 2)Usala et al J. Clin. Invest. 85:93 (1990) 3)Usala et al. J. Clin. Endocrinol. Metab. in press (1991) 4) Sakurai et al. P.N.A.S. 86:8977 (1989) 5)Usala et al. Biochem. Biophy Res. Commun. 171: 575 (1990)

The Regulation of TSH, TRH and Growth Hormone

L 036 THYROID HORMONE INHIBITION OF TSH GENE EXPRESSION REQUIRES PROTEIN INTERACTION WITH SPECIFIC DNA SEQUENCES INDEPENDENT OF PROMOTER POSITION, Frances E. Carr, Department of Clinical Investigation, Walter Reed Medical Center, Washington, DC 20307-5001
In previous gene transfer studies we have demonstrated that tissue specific thyroid hormone suppression of rat TSHβ subunit gene expression requires cis-acting elements in close proximity to transcriptional start site 2 (TSS2); -17 to +27. To determine the role of these sequences in TSHβ gene expression, we first examined protein binding activity. Gel mobility shift assays confirm the binding of nuclear proteins to DNA sequences -17 to +27. DNAse I footprinting resolved protected regions including +11 to +27 (CGCCAGTGCAAAGTAAG). Using transient transfections, we found the sequence +11 - +27 confers thyroid hormone sensitivity (negative regulation) to the thymidine kinase (TK) promoter when inserted as a single copy -125 to the transcriptional start site as well as in the native position (+11). In contrast, GH TRE (TCAGGTCATGACCTGA) confers positive regulation upstream of the TK promoter. Point mutations introduced into this region result in a selective loss of protein binding as measured by gel shift and DNAse I footprinting. Corresponding to this loss of protein binding is a loss of T_3 sensitivity. Co-transfection of a thyroid hormone receptor and TSHβ plasmids into either JEG3 or COS-1 cells confirmed the thyroid hormone receptor mediated activity. A 75-90% decrease in transcriptional activity is mediated by this sequence as also determined by an in vitro transcription assay. Sequences -17 to +27 also confer thyroid hormone regulation but are less potent. In summary, a 17 bp DNA sequence of the rat TSHβ exon 1 confers negative regulation by thyroid hormone both 5' and 3' to the transcriptional start site.

Molecular Approaches to the Study of Thyroid Hormone Action

Poster Session

L 100 POTENTIAL AUTOREGULATION OF THYROID HORMONE RECEPTOR TRANSCRIPTIONAL ACTIVITY BY TRUNCATED RECEPTOR PROTEIN. J. Bigler, W. Hokanson, and R.N. Eisenman. Division of Basic Sciences, Fred Hutchinson Cancer Research Center, 1124 Columbia St., Seattle WA 98104.

In avian embryonal erythroid cells *c-erbAα* is expressed as a nested set of at least four proteins, which have a common C-terminus. They appear to arise from translational initiation at internal, in-frame AUG codons, as shown by deletion and point mutations. We have demonstrated that all the forms bind hormone with high affinity, but only the two larger ones are able to bind to a thyroid hormone response element (TRE). They do so as dimers, since we observe exclusively a DNA-protein complex with a mobility corresponding to two ErbA proteins bound to the TRE.

In transactivation experiments the smaller, non-DNA-binding ErbA forms were found to inhibit the transcriptional activation by full-length ErbA. The same effect can be observed in the presence of excess thyroid hormone, ruling out inhibition by competition for ligand. We also found that truncated ErbA proteins can prevent the full-length form from binding to a TRE in gel mobility shift assays. Several mutations that we introduced into the putative dimerization/ligand binding domain lessened the inhibitory effect of truncated ErbA proteins in transactivation experiments as well as gel mobility shift assays.

Our results support the hypothesis that truncated ErbA proteins, which are expressed in vivo, can interfere with transcriptional activation by full-length ErbA through the formation of ErbA heterodimers, which are unable to bind to the response element.

L 101 *Abstract Withdrawn*

L 102 AN ESSENTIAL ROLE OF DOMAIN D IN THE HORMONE BINDING ACTIVITY OF HUMAN β1 THYROID HORMONE NUCLEAR RECEPTOR, Sheue-yann Cheng and Kwang-huei Lin, Laboratory of Molecular Biology, NCI, NIH, Bethesda, MD 20892

The human placental thyroid hormone nuclear receptor (h-TRβ1) could be divided into four functional domains: A/B, C, D and E. The E domain was thought to bind thyroid hormone. To evaluate whether domain E alone is sufficient to bind 3,3',5-triiodo-L-thyronine (T_3), a series of deletion mutants were constructed. The mutants were expressed in *Escherichia coli* and the expressed proteins were purified. Analysis of T_3 binding affinity and analog specificity of the purified truncated h-TRβ1 indicated that domain E alone did not have T_3 binding activity. Extension of the amino terminal sequence of domain E to include part of domain D (Lys^{201}-Asp^{456}) or the entire domain D (Met^{169}-Asp^{456}) yielded mutants with Ka's for T_3 of 0.5×10^9 M^{-1} and 0.8×10^9 M^{-1}, respectively. Further extension of the amino terminal sequence to include domain C yielded a mutant with a Ka similar to that of the wild type h-TRβ1 (Ka = 1.5×10^9 M^{-1}). Furthermore, mutant (Met^{169}-Asp^{456}) binds to Tripro, D-T_3, L-T_4 and r-T_3 with 307, 37, 7, and 0.1%, respectively, of the activity of L-T_3. This order of analog affinity is similar to that of the wild type h-TRβ1. These results indicate that domain D is essential for hormone binding activity. In contrast, A/B domain is not required for T_3 binding activity. Deletion of the last eight carboxyl amino acids abolished completely the T_3 binding activity of the mutant (Met^{169}-Asp^{456}). Thus, domain D is essential for domain E to function as a hormone binding domain.

Molecular Approaches to the Study of Thyroid Hormone Action

L 103 CARDIOACTIVE COMPETITORS FOR THYROXINE BINDING: CRYSTAL STRUCTURE OF TRANSTHYRETIN-MILRINONE COMPLEX. Vivian Cody, Andrzej Wojtczak and Joe Luft, Medical Foundation of Buffalo, 73 High St., Buffalo, NY 14203, USA.

Recent structure-activity data show that many pharmacological agents are strong competitors with thyroxine (T_4) for hormone binding sites on serum transport proteins and that this competition can interfere with their pharmacological actions. Milrinone, [2-methyl-5-cyano-(3,4'-bipyridin)-6(1H)-one], is a positive cardiac inotropic agent recently shown to have thyromimetic activity in vitro in a rabbit myocardial membrane Ca^{2+}-ATPase system. These data revealed that milrinone displaced T_4 from transthyretin (TTR), whereas amrinone, the 2-H-5-amino analogue, had less than 5% milrinone's activity as an inhibitor of T_4 binding. Computer graphic modeling of milrinone binding to the T_4 site in TTR revealed that it best occupied this site when the 5-cyano-4-keto pyridine ring overlapped the phenolic ring of T_4, placing the electronegative cyano group in the 3'-iodo position. The 5-amino group of amrinone does not share this characteristic. To understand how competitors recognize thyroid hormone binding sites on TTR, the crystal structure determination of TTR-competitor co-crystallized complexes are being investigated. We report preliminary results for a TTR-milrinone complex which crystallizes with two independent TTR monomers in the asymmetric unit; isomorphous with the native structure reported by Blake and coworkers. Structural data reveal electron density deep in the binding channel consistent with the forward binding of T_4, and in agreement with the computer models. These data show that although the 5-cyano pyridine ring is positioned deeper in the channel than T_4, it too fits in a hydrophobic pocket near that of the 3'-iodo atom. Supported in part by DK 41009.

L 104 BINDING OF THYROID HORMONE RECEPTOR TO T_3 RESPONSE HALF-SITES, Donna E. Crone, Hyoung Soon Kim and Stephen R. Spindler, Department of Biochemistry, University of California, Riverside, CA 92521
We have shown that α and β T_3 receptors (TRs) bind specifically and with high affinity to the 10 base pairs immediately 3' of the rat growth hormone (rGH) TATA box. Unlike previously identified T_3 hormone response elements (TREs), this element consists of a single half-site for TR binding. To extend understanding of receptor-DNA binding, we examined the TR interaction with half-sites within the previously identified rGH TREs. The half-site bound with highest affinity by TR was 5'-CTGAGGTAACTT-3'. The 5'-flanking and intronic rGH TREs are composed of one strong and one or more weak half-sites for TR binding. Further, in the intact 5'-flanking TRE, the weaker site(s) is cis-dominant with respect to receptor binding; affinity for direct or inverted repeats is less than affinity for the stronger half-site. Functional analysis of cis-promoter activation by individual half-sites is in progress.

L 105 EFFECTS OF TRIIODOTHYRONINE (T3) ON TERMINAL DIFFERENTIATION OF RAT CORTICAL NEURONS CULTURED IN A SYNTHETIC MEDIUM, Italia Di Liegro, Daniele Castiglia, Carlo Di Liegro, Lidia Bonfanti and Alessandro Cestelli, Dipartimento di Biologia Cellulare e dello Sviluppo, University of Palermo, Palermo, Italy.
It has been known for a long time that thyroid hormones exert a profound effect on mammalian brain development; however, the molecular basis of this action remains largely unknown, mainly because of the great complexity of the Central Nervous System. We have used a serum-free synthetic medium,which selectively substains survival of rat cortical neurons, to study some of the biosynthetic events which characterize neuronal terminal differentiation.We have previously reported that the addition of T3 to neuronal cultures induces a shortening of the chromatin repeat length which resembles the natural one. We have then studied the effect of T3 on the synthesis of nuclear proteins and the expression of the mRNAs which encode different variants of T3 nuclear receptors (c erbA proteins). In particular we report that: 1) T3 stimulates the turnover of nuclear proteins, with a more evident effect on the non-histone component; 2) for the entire period of culture the mRNA encoding the α2 variant (which is unable to bind T3) of T3 nuclear receptors is the predominant form; this finding suggests that the α2 predominance in the brain is settled very early during brain development.

L 106 CORRELATION OF THE DEGREE OF THYROID HORMONE RECEPTOR-INDUCED DNA BENDING WITH FUNCTIONAL RESPONSIVENESS OF THE RESPONSE ELEMENT.

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The human chorionic somatomammotropin (hCS) promoter contains a functional triiodothyronine (T_3) response element (TRE) between nucleotides (nts) -65/-44. The corresponding region of the homologous human growth hormone (hGH) promoter contains three sequence differences that result in: **(i)** loss of T_3 responsiveness, **(ii)** reduced T_3 receptor binding affinity (ca. 3- to 5-fold) and **(iii)** reduced T_3 receptor-induced DNA bending (ca. 50%) as assessed by circular permutation analysis of the binding sites. Introduction of mutations corresponding to the hGH sequences in the hCS -65/-44 binding site at nt -55 or nts -50 and -48 abolishes T_3 responsiveness. Circularly permuted DNA fragments containing the mutated hCS -65/-44 TREs also exhibit a 30 - 60% reduction in the degree of T_3 receptor-induced DNA bending. These data demonstrate that: **(i)** the T_3 receptor induces DNA bending upon binding to the TRE and **(ii)** the degree of bending is correlated with functional responsiveness. These results suggest that the conformation of the DNA in the receptor-TRE complex influences the binding affinity of the receptor and/or the functional activation state of the receptor.

L 107 THYROID HORMONE DECREASES THE STEADY STATE LEVELS OF mRNA FOR PRO α_2 (I) COLLAGEN IN THE RAT MYOCARDIUM AND IN CARDIAC FIBROBLASTS IN CULTURE

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Little is known about the regulatory effects of thyroid hormone on cardiac interstitium. In the present study we examined the effects of thyroid hormone on myocardial collagen biosynthesis. Adult male Sprague Dawley rats were treated intraperiotonealy with L-thyroxin (10μg/100g body weight). Rats were sacrificed at 2 hr, day 1, day 2, day 3, day 6 and 2 weeks following treatment. Northern hybridization analysis was performed on RNA extracted from total ventricular tissue. It was shown that after 2 hours of treatment, the abundance of mRNA for pro α_2(I) collagen in treated hearts decreased by 53% ($p < 0.05$) and reached to the lowest level (60% decrease, $p < 0.02$) at day 1, remained diminished at day 3 and gradually returned to the normal levels at 12 days of treatment. Direct effect of thyroid hormone on cardiac fibroblasts was examined by treatment of these cells in culture (10 nM) for 24 hours. Results of Northern hybridization showed a 33% ($p < 0.005$) decrease in the abundance of mRNA for pro α_2 (I) collagen in treated fibroblasts compared to that in control untreated cells. We therefore conclude that thyroid hormone may be considered as a regulator of myocardial collagen biosynthesis.

L 108 **INTERACTION of E. COLI EXPRESSED α AND βc-erbA PRODUCTS WITH CIS-ACTING ELEMENTS OF THE HUMAN β- MYOSIN HEAVY CHAIN GENE PROMOTER,**

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The human β-myosin heavy chain (β-MHC) gene is under negative transcriptional regulation by 3,5,3'-triiodo-L-thyronine (T_3). Functional analysis of the β-MHC gene promoter reveals major regions of repression by T_3 on both sides of a strong positive element. To specifically localize the T_3 responsive elements (TREs), we have expressed α and βc-erbA products in E.coli, and studied their interactions with β-MHC promoter sequences using mobility shift assays and footprint analyses. The results demonstrate four TREs positioned at -444/-463 (TREβ1), -384/-435 (TREβ2), -58/-78 (TREβ3), and -20/-51 (TREβ4) from the CAP site. TREβ1 contains a nanomeric purine-rich sequence, 5'-CAGG(G)AGGA-3', similar to a strong TRE in the α-MHC gene. TREβ2 and TREβ4 contain octameric elements in opposite orientations. TREβ3 is identical to the consensus sequence of a TRE found in the α-TSH gene, 5'-(G/A)GTG(G/A)G-3'. TREβ3 is located between CAAT and TATA and TREβ4 overlaps the TATA box. These results suggest that multiple TREs coordinately down-regulate expression of the human β-MHC gene.

L 109 HYPOTHYROID INDUCTION OF LIPOGENESIS IN BROWN ADIPOSE TISSUE IS MEDIATED BY THE SYMPATHETIC NERVOUS SYSTEM, Hedley C. Freake and Wen-Jin Yeh, Department of Nutritional Sciences, University of Connecticut, Storrs, CT 06269-4017.

We have previously shown that the levels of mRNA-S14, which encodes a protein thought to play a role in fatty acid synthesis, and lipogenesis itself are higher in brown adipose tissue (BAT) taken from hypothyroid as opposed to euthyroid rats. This inverse regulation is opposite to that seen in liver in the same animals. The sympathetic nervous system is an important regulator of BAT function and we have investigated whether an intact nerve supply is required for the hypothyroid response. Two weeks prior to experimentation, the left interscapular BAT pad was surgically denervated, leaving the right pad intact as a control within the same animal. On the intact side, fatty acid synthesis, assessed by tritiated water incorporation, was higher in hypo- than in euthyroid rats (88.4 $\pm$ 26.6 vs 45.2 $\pm$ 6.5 μg H inc./g/h). Denervation resulted in a greater decrement in lipogenesis in hypothyroid animals such that levels fell below euthyroid BAT (7.4 $\pm$ 1.3 vs 21.2 $\pm$ 2.2 μg H inc./g/h). Furthermore, treatment of hemi-denervated hypothyroid animals with triiodothyronine resulted in a dose dependent increase in fatty acid synthesis in denervated BAT, which paralleled that seen in liver. Low doses of triiodothyronine resulted in a decrease in lipogenesis on the intact side, while high doses were without effect. mRNA-S14 levels were also quantitated and found to parallel the measurements of fatty acid synthesis, again confirming the association of this sequence with the lipogenic process. These results suggest that lipogenesis and mRNA-S14 expression in BAT are stimulated both by the sympathetic nervous system and by thyroid hormone. The apparent stimulation by hypothyroidism may be a consequence of decreased thyroid-dependent thermogenesis in tissues other than BAT. This will lead to cold stress and result in an increased sympathetic signal to BAT in an attempt to make up the decrement in heat production.

L 110 ONTOGENY OF C-ERBA mRNA AND THYROID HORMONE RECEPTOR IN CHICK EMBRYOS. Alain Giguère, Suzanne Fortier, Jean-Guy Lehoux and Diego Bellabarba, Endocrine Laboratory, Sherbrooke University School of Medecine, Sherbrooke, Québec, CANADA, JIH-5N4.

Embryo development is associated with significant changes of T3 binding in target organs. In order to understand if such changes are regulate at the level of the syntheses of their mRNA, in the present study we have compared the ontogenic pattern of mRNA of c-erbAs to that of receptor binding in liver and brain. Total RNA was extracted from liver and brain of chick embryo of 9 to 19 days of age by technique of Chirgwin and al. (Biochemistry, 18, 5294, 1979) and analyzed by Northern blot hybridization (Strait et al., J.B.C., 265, 10514, 1990) using cDNA probes from chick embryo and rat. After washing and autoradiography of the blots, the identified mRNAs spots were quantitated by video densitometry. With the α1 probe from chicken or rat we identified a major lane corresponding to 4.5 kb and minor ones of 3.2 and 2.6 kb. With both ß1 probes we observed a signal of 9.3 kb, whereas with rat α2 a 1.9 kb signal was evident. The ontogenic patterns revealed a slight increase of the 4.5kb signal in brain and a more sharp rise in liver particularly from 9 to 12. The mRNA signal of ß1 in the brain doubled from 17 to 19, whereas it changed little in the liver. As for the 1.9Kb mRNA signal, it did not show any significant change in brain and only a slight raise in liver. T3 binding by the hepatic receptors rose progressively during embryogenesis and this pattern could correlate with those of α1 and α2 mRNAs. In the brain, however, the T3 binding rose sharply from 9 to 12 and the fell to low value at day 19, a pattern which did not correlate with any of c-erbA mRNAs. There fore these data indicate little correlation between ontogeny of c-erbA mRNAs and T3 receptor binding, particularly in brain, and suggest that, during chick embryo development of the brain, the ontogeny of T3 receptors is regulated at levels others than the mRNA synthesis.

L 111 ALTERED LEVELS OF *erb*A RNA TRANSCRIPTS IN NEOPLASTIC RODENT CELLS, Duane L. Guernsey and Catherine K.L. Too, Department of Pathology, Dalhousie University Faculty of Medicine, Sir Charles Tupper Medical Building, Halifax, Nova Scotia, Canada B3H 4H7

We have previously demonstrated that thyroid hormone is a necessary co-factor for the initiation of neoplastic transformation *in vitro*. Depleting thyroid hormones from the culture media either eliminated or dramatically suppressed transformation of rodent cells by x-rays, chemicals, viruses or DNA-transfection. Additionally, we found that thyroid hormone regulation of inducible pathways was lost when the cells were transformed. Transformation of the cells was associated with a decrease in triiodothyronine (T3) binding to isolated nuclei. Our current research is to investigate the expression of the multiple *erb*A transcripts in neoplastic cells compared to normal counterparts. We hypothesize that the decreased T3 binding and loss of T3 responsiveness in transformed rodent cells may be due to either 1) a decrease in the level of *erb*A-α1 and *erb*A-β transcripts (physiologic receptors), or 2) an increase in the level of the *erb*A-α2 transcript (antagonist receptor). We have found a 1.5-2.0 fold increase in the steady-state level of the *erb*A-α2 transcript in transformed cells compared to normal counterparts in the proliferative state. Interestingly, in the normal cells the level of the *erb*A-α2 transcript increases when the cells are growth arrested by confluence. While rat cell lines express the *erb*A-β transcript, we were unable to detect the RNA in mouse cell lines. We suggest that the altered levels of the *erb*A transcripts in neoplastic cells may be involved in the expression of the neoplastic phenotype.

L 112 NEGATIVE GENE REGULATION BY THYROID HORMONE RECEPTORS, Thomas Hermann, Xiao-kun Zhang, Maty Tzukerman, Ken W. Wills, Gerhart Graupner, and Magnus Pfahl, La Jolla Cancer Research Foundation, La Jolla, CA 92037.

Thyroid hormone receptors (TR) belong to a large family of intracellular transcriptional regulatory proteins that also include the retinoic acid receptors (RAR) and the estrogen receptor (ER). TRs are encoded by at least two genes, the c-erbA genes α and β. From each gene different isoforms can be produced. Some of these isoforms activate transcription from thyroid responsive elements (TRE) in response to hormone (T_3). Heterologous receptors like the RARβ and the ER are also efficient TRE-activators. These findings suggest that TRs regulate gene expression via responsive elements that have a broad receptor specificity. In addition to the positive effects on gene regulation, TRs exert negative control by repressing TRE-regulated genes. Studies using transient transfection and gel shift assays show that the mechanisms of repressing are different for different TR-isoforms. The active (hormone-inducible) isoforms TRα1 and TRβ bind tightly to the TRE in the absence of hormone thereby silencing the respective genes and preventing them from being activated by heterologous receptors. This competitive mechanism greatly increases the specificity of the TRE. TRα2 is an alternative splice-form that differs from TRα1 in the carboxyterminal region. This results not only in the lack of T_3-binding and gene activation but also in the inability to form homodimers. Therefore, TRα2 binding to the TRE is extremely weak. However, TRα2 represses active TRs, the RARβ and the ER. Immuno-coprecipitation analysis indicate that this function is based on a mechanism involving hetero-oligomer formation. The biological role for TRα2 thus could involve repression of genes, induced by leaking expression of active receptors.

L 113 THYROMYMETIC EFFECT OF PEROXISOMAL PROLIFERATORS IN RAT LIVER, R. Hertz and J. Bar-Tana, Department of Biochemistry, Hebrew University School of Medicine, Jerusalem 91010, Israel.

Amphipathic carboxylates of varying hydrophobic backbones which act as peroxisomal proliferators (aryloxyalkanoic acids, methyl-substituted dicarboxylic acid) induce in euthyroid or thyroidectomized rats as well as in rat hepatocytes cultured in T3-free media liver activities classically considered as thyroid hormone-dependent (malic enzyme, mitochondrial α-glycerophosphate dehydrogenase, glucose-6-phosphate dehydrogenase and S14). The dose required in vivo for the thyromimetic effect of peroxisomal proliferators was 10^3 fold higher as compared to triiodothyronine. Similarly, peroxisomal proliferators were active in culture in the range of 10^{-6}-10^{-4} M as compared to 10^{-9} M for triiodothyronine. Their maximal inductive capacities were however similar to or exceeded that of triiodothyronine. The thyromimetic effect of peroxisomal proliferators was only partially correlated with their capacities as inducers of liver peroxisomal enzymes. The thyromimetic effect with respect to liver malic enzyme and S14 resulted from an increase in their mRNAs content. The increase in liver S14 mRNA was accounted for by transcriptional activation of the S14 gene. Triiodothyronine binding to isolated liver nuclei or nuclear extract was competitively displaced by some but not all nonthyroidal inducers of the above liver activities. In contrast to the thyromimetic effect induced in liver cells, no increase in growth hormone mRNA was observed in cultured GH1 pituitary cells incubated in the presence of nonthyroidal amphipathic carboxylates. The characteristics of the thyromimetic effect of amphipathic carboxylic peroxisomal proliferators may indicate that these agents may act as transcriptional activators of thyroid hormone-dependent genes in the rat liver.

L 114 EFFECT OF THYROID HORMONES ON GLUCOKINASE GENE EXPRESSION IN DIABETIC RATS, W. Höppner, H. J. Seitz, S. Frischmann, R. Piepho, Institut f. Physiologische Chemie, University of Hamburg, Germany

We have recently shown that hepatic glucose utilisation is stimulated by thyroid hormones via the induction of glucokinase at the transcriptional level. T3 acts synergistically on the insulin-mediated induction of glucokinase after carbohydrate refeeding. The present study was set out to investigate the effect of thyroid hormones on Glucokinase gene transcription in the abscence of insulin. In diabetic rats the amount of glucokinase mRNA is reduced to basal levels and insulin administration is necessary to obtain a significant increase in glucokinase mRNA upon glucose refeeding. Hyperthyroidism, produced by daily injection of thyroxine for 7 days in non-diabetic rats, led to a 3-fold inrease in glucokinase mRNA levels during starvation. In diabetic rats the increase in glucokinase gene transcription after carbohydrate feeding was significantly higher in hypertyroid rats than in euthyroid diabetic animals. Run-off transcription assays demonstrate, that the rate of glucokoinase gene transcritption is accelarated in hyperthyroid starved rats and in carbohydrate-fed diabetic rats implying that T3 can activate glucokinase gene transcription independently from the action of insulin.

L 115 THE C'-TERMINAL INTERACTION DOMAIN OF THE THYROID HORMONE RECEPTOR CONFERS THE ABILITY OF THE DNA SITE TO DICTATE POSITIVE OR NEGATIVE TRANSCRIPTIONAL ACTIVITY, Holloway, J.F., Glass, C.K., Adler, S., Nelson, C.A., & Rosenfeld, M.G. Cellular and Molecular Medicine, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0648.
To investigate mechanisms responsible for positive and negative transcriptional control, we have utilized two types of promoters that are differentially regulated by thyroid hormone (T_3) receptors. Promoters containing the palindromic T_3 response element TCAGGTCA TGACCTGA are positively regulated by the thyroid hormone receptor after the administration of T_3, while otherwise identical promoters containing the estrogen response element TCAGGTCA CTG TGACCTGA can be regulated negatively; converse effects are observed with the estrogen receptor. We describe evidence that the transcriptional inhibitory effects of the T_3 and estrogen receptors on the estrogen or T_3 response elements, respectively, are imposed by amino acid sequences in the C'-terminal region that co-localize with dimerization and hormone binding domains, and that these sequences can transfer inhibitory functions to other classes of transcription factors. Removal of the C'-terminal dimerization and hormone-binding domains of either the alpha T_3 or estrogen receptors permits each receptor to act constitutively to enhance transcription on both T_3 and estrogen response elements. It is therefore suggested that protein-protein interactions between receptor C'-termini limit the subset of DNA binding sites on which transcriptional activation occurs.

L 116 LONG-CHAIN FATTY ACIDS AND FATTY ACYL-CoAs ARE POTENT INHIBITORS OF THE NUCLEAR THYROID HORMONE RECEPTOR, Akira Inoue, Qiulin Li, Naoki Yamamoto, Toshiko Uchimoto, Yuji Morisawa and Seiji Morisawa, Department of Biochemistry, Osaka City University Medical School, Abeno-ku, Osaka, Osaka 545, Japan
Long-chain fatty acids are important fuels in thermogenesis. The synthesis of fatty acids in lipogenic tissues and their release from fat tissue are positively controlled by thyroid hormone (T_3). We report that T_3 action is, in turn, inhibited by fatty acids and fatty acyl-CoAs: they are potent inhibitors of the thyroid hormone receptors, isolated from the nuclei of various rat tissues. Fifty per cent inhibition of T_3 binding by hepatic receptors was observed at oleic acid and oleoyl-CoA concentrations of 2.8 and 1.3 uM, and their affinities for the receptor (Ki) were 1.0 and 0.45 uM, respectively. They also enhanced dissociation of the preformed T_3-receptor complex. Their action was competitive for the hormone binding site, resulting in a reduction in the receptor's affinity for T_3. In HTC rat hepatoma cells in culture, fatty acids added to the medium reached the nucleus and inhibited nuclear T_3 binding. T_3 binding of the cells was reversibly restored in fresh medium free of added fatty acids. These observations suggest that fatty acids and acyl-CoAs modulate the binding of the thyroid hormone to its nuclear receptors. Thus, it is likely that while T_3 promotes fatty acid synthesis, an increased concentration of fatty acids feedback and inhibit the synthetic process at the level of nuclear T_3 receptor.

L 117 CEREBELLAR DEVELOPMENTAL ALTERATIONS IN APO E AND TRANSFERRIN GENE EXPRESSION IN PTU-TREATED HYPOTHYROID RATS D.M. Jaworski, N.C. Mills and C.M. Craft* Texas Woman's Univ., Dept. of Biology, Denton TX, 76204 and *UT Southwestern Medical Center, Dept. of Psychiatry, Dallas TX, 75235.
Thyroxine alters the developmental expression of several genes. We investigated cerebellar gene expression and protein synthesis of apolipoprotein E (apo E) and transferrin (T_f) in rats treated with the goitrogen propylthiouracil (PTU) to induce hypothyroidism. Known central nervous system alterations in this model include abnormal cellular morphology, migration and synaptogenesis. Poly A^+ selected RNA from pooled cerebella of 1,10,20,30 and 60 day postnatal male rats was in vitro translated and examined by gel electrophoresis (PAGE). Apo E and T_f translated proteins were selectively immunoprecipitated, washed with Pansorbin and separated by PAGE. Northern analysis of each timepoint was examined with cDNA probes for apo E and T_f. To further quantitate mRNA expression, labelled polymerase chain reaction was performed for each mRNA. Three experimental groups were tested. First, PTU treatment began in utero and ceased at postnatal day 10. As PTU levels decrease and thyroxine is synthesized, induction of both apo E and T_f expression occurred. Second, when PTU treatment was continued to 60 days postnatal, expression of both was inhibited. Finally, when PTU treatment began at postnatal day 10, apo E and T_f expression declined. Our results suggest that thyroxine alters cerebellar developmental expression of apo E and transferrin.

L 118 EXERCISE-INDUCED ALPHA MYOSIN HEAVY CHAIN GENE EXPRESSION IN THE HYPOTHYROID RAT HEART, Harvey L. Katzeff, Kaie Ojamaa and Irwin Klein. North Shore University Hospital-Cornell University Medical Center, Manhasset, NY 11030
Hypothyroidism in rats suppresses food intake and both skeletal muscle and cardiac muscle protein synthetic rates. In cardiac muscle, there is a switch to the V_3 myosin isoform with complete suppression of alpha myosin heavy chain (MHC) gene expression. To determine whether thyroid hormones are a necessary cofactor for exercise-induced muscle growth, we studied MHC gene expression using specific oligonucleotide probes in skeletal and cardiac tissue. The experimental protocol compared 3 groups: 1. Hypothyroid (TX) 2. Exercise + Hypothyroid (TX+EX) 3. Overfed + Hypothyroid (TX+OF). RNA was extracted from cardiac and skeletal muscle at sacrifice and α and β MHC expression determined by northern blot.

	Tx	Exercise +TX	Overfed + TX
Body Weight-g	240 ± 28	229 ± 9	300 ± 18*
Heart/Body Weight * 100	1.93 ± .19	2.56 ± .37**	2.13 ± .31
alpha MHC	—	++	—
beta MHC	++	+++	++

n=5 per group; Mean ± SEM; — = absent; + = present; *= $p<0.02$; **= $p<0.01$
Exercise induced the expression of α MHC de _novo_ in the hypothyroid heart in association with cardiac hypertrophy. In contrast, the expression of myosin cardiac isoforms were not altered by overfeeding despite a comparable increase in heart size. These data suggest that thyroid hormones are not obligatory for the expression of the alpha isoform of MHC. Exercise-mediated hemodynamic effects on the cardiac MHC gene expression are mediated independently from thyroid hormones.

L 119 THYROID HORMONE INHIBITION OF THE ESTROGEN RESPONSE: PROTEIN INTERACTIONS OR RECEPTOR-RECEPTOR COMPETITION FOR DNA SITES? Peter J. Kushner, Gabriela N. Lopez, John D. Baxter, and Tom Lavin, Metabolic Research Unit, University of California, San Francisco, CA 94143
Thyroid hormone inhibits the estrogen response mediated by the vitellogenin A2 gene estrogen response element (ERE) in cells with both estrogen and thyroid hormone receptors. To determine whether "competition" for the common receptor binding site or another mechanism such as squelching, or direct protein interactions between the receptors could account for the inhibition, we transfected reporter genes bearing the vitellogenin ERE along with expression vectors for the two receptors into Hela cells. The ERE mediates a modest thyroid hormone response (5 fold) and a strong estrogen response (500 fold) which in turn is severely inhibited by thyroid hormone. Thyroid hormone inhibition of the estrogen response varies from nil to 96% with increasing amount of TR expression vector. Moreover, while neither the glucocorticoid receptor (GR) nor an estrogen receptor with a GR DNA binding domain is inhibited at a glucocorticoid response element by the TR plus T3, a glucocorticoid receptor with an estrogen receptor DNA binding domain is inhibited from activating at an ERE by TR plus T3. Gel retardation assays of the two receptors indicate that they compete with one another for binding to the vitellogenin hormone response element, and either one or the other receptor exclusively binds with no evidence for co-binding. Although these observations seem to support the competition model, we are currently performing some critical tests which should provide an unambiguous answer.

L 120 DOMINANT-NEGATIVE EXPRESSION VECTORS OF RETINOIC ACID RECEPTORS, Elwood Linney, Amy Espeseth, Susan Kandel, and Wayne Balkan, Department of Microbiology and Immunology, Box 3020, Duke University Medical Center, Durham, NC 27710
The retinoic acid receptors(RARs) are ligand-inducible transcription factors which bind to thyroid receptor elements(TREs) and retinoic acid receptor elements(RAREs). We are using a biological assay system to investigate the function and specificity of RARs during differentiation. We have previously shown(Espeseth et al, 1989, Genes and Development _3_ 1647)that overexpression of a vector expressing the A,B,C, and part of the D domain of the hRAR-alpha fused to the bacterial beta-galactosidase gene inhibited the retinoic acid induced differentiation of F9 embryonal carcinoma cells. We have found that vectors containing analogous regions of mRAR-beta, -gamma-1(A) and -gamma-2(B) also produce retinoic acid resistant clones of F9 embryonal carcinoma cells. We will report on the molecular phenotype of these clones and how we can separate dominant negative mechanisms through vectors containing just the A and B domain of hRAR-alpha or the C and part of the D domain of hRAR-alpha.

L 121 EFFECTS OF THYROID HORMONE ON THE LIPOLYTIC RESPONSE OF NEONATAL MONOSODIUM-L-GLUTAMATE (MSG) TREATED RATS, W.K. Liu and C.C. Wong*, Departments of Anatomy and Physiology*, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong.
In vitro lipolytic response of epididymal adipocytes to isoproterenol (ISO) and adrenocorticotropin (ACTH) stimulation was significantly reduced in neonatal MSG-treated rats. This activity could not be restored by a short-term thyroid hormone treatment, i.e., a single bolus injection or three daily injection of 150 ug T_3/kg/d, unless the MSG-rats were given 10 daily doses of T_4 at 20 or 100 ug/kg/d. A reduction of β-adreno-receptor was observed in these epididymal adipocytes, but neither dibutyryl-cAMP nor isobutylmethylxanthine could recover their normal lipolytic responses. In the presence of adenosine deaminase (AD), cultured adipocytes increased their lipolytic activity, which could further be enhanced in adipocytes from T_3-treated rats. Synergistic effects of ISO and AD on lipolysis were also demonstrated in adipocytes, indicating that neonatal MSG-treatment in rats interferes with the response of adipocytes to lipolytic stimuli, and only a long-term thyroid hormone treatment could restore this activity.

L 122 ANTAGONISM OF BOTH POSITIVE AND NEGATIVE THYROID HORMONE EFFECTS ON GENE EXPRESSION BY AMIODARONE, Gabriela N. Lopez, John D. Baxter, and Peter J. Kushner, Metabolic Research Unit, University of California, San Francisco, CA 94143
An antagonist for thyroid hormone action at the receptor level has not been established but amiodarone, a drug with some similarities to thyroid hormone (T3), is a good candidate. We investigated the effects of amiodarone on the response of transfected reporter genes bearing thyroid hormone response elements (TREs) that confer both positive and negative responses to T3. When a CAT reporter gene bearing a synthetic positive T3 response element (TREpal) was transfected into Hela cells along with an expression vector for the human beta T3 receptor (TR) the induction with T3 was largely blocked by amiodarone. Complete blockade, however, was only seen at high amiodarone concentrations which are partly toxic. When a tk:CAT reporter bearing the combined ERE-TRE from the Xenopus vitellogenin A2 gene was transfected along with expression vectors for the estrogen receptor and TR in a one to ten ratio, we observed a dramatic induction with estrogen that was inhibited by T3. Addition of increasing concentrations of amiodarone completely relieved the negative effects of T3. These observations strengthen the notion that amiodarone binds the TR without activating the receptor and prevents T3 binding and subsequent activation.

L 123 DIFFERENTIAL EXPRESSION AND REGULATION OF α AND β THYROID HORMONE RECEPTOR mRNAs IN RAT BRAIN CELL CULTURES, Puymirat J., Turgeon C., Lhérault S. and J.H. Dussault. Department of ontogenesis and molecular Biology, CHU Laval, 2705 Bd Laurier, Ste-Foy, Québec, G1V 4G2.

There is several evidences that the cellular products of the protooncogene erbA code for two families of T3 receptors (TR), TRα and β. Both type of TR mRNAs are present in the adult rat brain. However, the cellular localization of these mRNAs remains unknow. By Northern analyses using specific oligonucleotides, we have studied the expression of each form of TR mRNAs in pure neuronal and astroglial cultures initiated from fetal rat cerebral cortex.
In neuronal cultures grown in serum-free medium, the rTRβ-specific probe detected RNAs of 6.0kb, while probes specific for rTR α1 and α2 hybridized to a 5- and 6 kb and to a 2.6kd RNAs, respectively. The rTRα2mRNAs were the predominant form of TR mRNAs. The rTRβ mRNAs were detected at the initiation of the culture and their level increased by 5.6 fold between day 8 and day 22. The rTRα1 RNAs remained stable between 8 and 15 days and decreased thereafter. During the first week, the level of the rTRα1 mRNAs was two fold higher than the level of the rTRβ RNAs, while the rTRβ became the predominant form of T3 binding proteins mRNAs thereafter. L-T3 added to the culture had no effect on the level of α1, α2 and β rTRmRNAs. In pure astroglial cultures grown in the absence of T3, the level of rTrα1 mRNAs was 5 fold higher than that of the rTRβ mRNAs whatever the age of the culture. L-T3 treatment significantly increased the level of rTRβ mRNAs without affecting the level of α1 and α2 mRNAs.
Conclusion. This study demonstrates:1) the presence of α and β TR mRNAs in neurons and in astroglial cells. 2) The rTRα1 mRNAs are the predominant T3 binding proteins mRNAs during the first week of neuronal cultures whereas the β mRNAs become the major form thereafter. 3) L-T3 regulate the expression of the rTRβ mRNAs in astrocytes but not in neurons.

L 124 THYROID HORMONE REGULATION OF S11/APOLIPOPROTEIN A1 GENE EXPRESSION. Jacques S. Romney, and Norman C.W. Wong, Depts of Medicine and Medical Biochemistry, U of Calgary, Calgary, AB, CANADA.
Previous studies have shown the rat liver S11 gene to be a useful model for studying the mechanisms of thyroid hormone action because of its 20-fold induction by L-triiodothyronine (T3). Recent studies showed that the nucleotide sequence of S11 cDNA is identical to the apolipoprotein A1 (Apo A1) mRNA from positions 419 to 859. The identity of S11 was confirmed by hybridizing duplicate northern blots containing total hepatic RNA from rats of hypo-, eu-, and hyperthyroid rats with radiolabelled authentic Apo A1 and S11 cDNA. Both probes hybridized to mRNA species of identical size and response to T3 demonstrating clearly that S11 and Apo A1 sequences were the same. To determine whether DNA sequences immediately upstream from the transcription start site of the S11/ Apo A1 gene play a role in mediating the effects of T3, we synthesized a 478 bp fragment, U-1 using the PCR. U-1 was inserted infront of a reporter 'G' free cassette (GFC) in the 5' to 3' [Apo-1-GFC(1)] or 3' to 5' [Apo-1-GFC(1R)] orientation and tested in a cell-free in vitro transcription assay. Incubation of the template DNA in reactions containing ^{32}P-UTP, and hepatonuclear extracts yielded radiolabelled RNA only when the U-1 fragment inserted was in the 5' to 3' but not the 3' to 5' orientation relative to the GFC. Since the mouse albumin and adenovirus major late promoter were active in all incubations, this indicated the importance of 5' to 3' orientation of U-1 DNA for transcription of the GFC. Activity of the the Apo-1-GFC(1) template in extracts from animals of various thyroid states was not significantly different amongst the 3 thyroid states. Furthermore, the addition of T3 to the incubation did not alter template activity. Next we used the gel-retardation assay to identify nuclear factors binding to U-1 DNA between nucleotides -131 to -190 that appeared to be critical for maximal Apo A1 expression. In summary, results confirm the identity of S11 to be the Apo A1 gene and T3 probably has a post-transcription effect in regulating activity of the gene. Hepatonuclear factors binding to -131 to -190 of U-1 DNA play an important role in regulating S11/Apo A1 gene expression.

L 125 **GENOMIC ORGANIZATION OF THE THYROID HORMONE RECEPTOR β GENES IN XENOPUS LAEVIS**

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We have analyzed the structure of the two thyroid hormone receptor β genes in amphibian X. laevis. The organization of most of their coding regions is similar. This comprises 6 exons within about 8 kb. Each of the two zinc fingers is encoded by a single exon, as is the case for other known members of the steroid hormone receptor super-family. The last exon of the coding region also contains at least 600 bp of the 3'-untranslated region (3'-UTR), which is about 8 kb. The sequences of the exons and 3'-UTRs of the two genes are highly homologous, whereas both the intron sizes and their sequences are very diverged from each other. In contrast to the simple structure of the coding region and 3'-UTR, the 5'-UTR and the extreme 5'-end of the coding region (2-47 aa) are encoded by as many as 8 exons. Complex alternative splicing of these exons can produce the different cDNA forms reported earlier. These 8 exons span at least 40 kb of the genome, although the exact size has not been determined. The cap sites of the mRNA have been mapped by a modified primer extension method and two cap sites were found for each of the receptor genes. These results suggest that the receptor gene expression is under complex regulation with alternative promoter usages as well as alternative splicing.

L 126 THYROID HORMONE STIMULATES mRNA LEVELS FOR THE Ca-ATPase OF SARCOPLASMIC RETICULUM IN NEONATAL SKELETAL MUSCLE AND L6 MUSCLE CELLS. Warner S. Simonides, Gerard C. van der Linden, Alice Müller and Cornelis van Hardeveld, Laboratory for Physiology, Free University, van der Boechorststraat 7, 1081 BT, Amsterdam, The Netherlands.
Thyroid hormone (T_3) regulates the level of Ca-ATPase in sarcoplasmic reticulum (SR) of skeletal muscle and heart. T_3-dependent increases in activity of this Ca pump largely account for the increase in energy turnover during contraction, and contribute to the T_3-dependent resting metabolic rate. Regulation of SR Ca-ATPase expression is therefore a factor in the thermogenic action of T_3. We now focus on the mechanism of action of T_3 and we have examined in more detail the neonatal development of SR Ca-ATPase activity in the rat, which was previously shown to be critically dependent on T_3. Levels of mRNA for the SR Ca-ATPase in fast muscle (gastrocnemius) were low at birth (day 0), but increased abruptly at day 6 to stabilize at a 5-fold higher level at day 21. The increase coincides with the start of endogenous T_3 production as well as with the previously found stimulation of Ca-ATPase activity. Daily subcutaneous T_3 injections started at day 2 induced a precocious stimulation of the Ca-ATPase mRNA level already within 6 hrs. Stimulation was 2-fold after 2 days, and a 5-fold higher plateau was reached after 10 days. Hypothyroid pups showed absolutely no stimulation of Ca-ATPase mRNA over a period of 7 weeks. T_3 treatment of these pups induced a normal pattern of mRNA stimulation and Ca-ATPase synthesis, even when started after 6 weeks. To investigate the possible involvement of other T_3-dependent systemic factors in the regulation of Ca-ATPase expression *in vivo*, we examined the continuous rat muscle cell line L6. The level of Ca-ATPase mRNA in differentiating L6 myotubes was stimulated 10-fold by T_3, corresponding with a similar increase in the level of Ca-ATPase synthesized. These results show the SR Ca-ATPase to be highly responsive to T_3, and indicate that T_3 acts, at least in part, at a pre-translational level.

L 127 DEVELOPMENTAL SWITCHING OF EXPRESSION OF mRNAs FOR N-TERMINAL VARIANTS OF THYROID HORMONE RECEPTOR β IN EMBRYONIC EYE. **Maria Sjöberg, Douglas Forrest and Björn Vennström, Department of Molecular Biology, CMB, Karolinska Institute, S-104 01 Stockholm, Sweden**

Thyroid hormones are essential for development of the vertebrate nervous system. Two types of thyroid hormone receptors (TRα and β), encoded by distinct genes, differ both in structure and expression. Expression of TRα mRNA is apparently ubiquitous whereas that of TRβ is restricted and is developmentally regulated. We have demonstrated striking differences in expression of TR genes in chick brain development with respect to timing and cell-specificity: TRβ mRNA was sharply induced in later maturing neural cells correlating with the known later period of hormone requirement, whereas TRα mRNA was already expressed in earlier developmental phases. In the early embryo before embryonic day 9 (E9), TRβ mRNA was relatively abundant only in the retina and *in situ* hybridisation localized expression in the outer neuroblastic layer. Furthermore RNase protection analyses revealed predominant expression of mRNA for a TRβ N-terminal variant (cTRβ2) in eye at E6, whereas later in development by E14, mRNA for the more common cTRβ (cTRβ1) predominated. The sequence of cDNA clones of cTRβ2 showed partial homology in the N-terminus to the rat TRβ2 which is expressed in adult rat pituitary. Early embryonic expression of specific TR forms before the well-described requirement for thyroid hormone later in development of the nervous system suggests wider developmental functions for TRs than may have been previously considered. We are analysing in detail early embryonic expression of cTRβ2 and also its trans-activating function compared to the previously characterized chick α and β TRs.

L 128 STRUCTURAL STUDIES OF A THYROID HORMONE RECEPTOR SINGLE "ZINC FINGER" DOMAIN

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Thyroid hormones can both induce and repress the expression of specific genes via a cascade of events beginning with recognition by a nuclear thyroid hormone receptor (T_3R). The T_3Rs belong to a superfamily of ligand-modulated transcription factors that contain two "zinc finger" motifs within their DNA binding domains. A 26 amino acid peptide has been synthesized that comprises the first "zinc finger" of the DNA binding domain of r-erbA-β-1, a T_3R. The peptide sequence contains the Cys-X_2-Cys-X_{13}-Cys-X_2-Cys motif. The reduced peptide has been studied using UV-VIS, CD and NMR spectroscopies and is found to bind to Zn^{2+} and Co^{2+} with high affinity at pH values greater than 5.0. In contrast to the recently published solution structure of the glucocorticoid receptor DNA binding domain (Science 1990, 249, 157), NMR studies indicate that the His residue situated in the X_{13} loop plays a role in metal binding of the T_3R peptide. CD studies indicate a small but reproducible decrease in the magnitude of (negative) ellipticity around 200 nm upon addition of $ZnCl_2$. This suggests either an increase in ordered secondary structure or a change in the aromatic contribution to far UV CD upon zinc binding. Solution conditions under which predominantly a single conformation of Zn-bound peptide exist have been identified by ^1H NMR, and 2-D NMR experiments are underway to define further the secondary structure and metrical parameters of metal ligation.

L 129 Sequence requirements of thyroid hormone receptor response elements (T3REs). **Gunilla Wahlström, Björn Vennström. Department of Molecular Biology, CMB, Karolinska Institute, Stockholm, Sweden.**

Thyroid hormones (T3, T4) produce diverse physiological and developmental effects. This control is mediated by the thyroid hormone receptors (TRs) which regulate transcription of target genes through specific response elements (T3REs). The T3REs behave as transcriptional enhancers which function independently of position and orientation but dependent upon presence or absence of ligand. They contain a consensus motif GGTCA which can occur as palindromic or direct repeats. We are currently determining the sequence and structural requirements of different T3REs with regard to binding of receptor and their function in TR-mediated transcriptional regulation of T3RE-reporter gene constructs. Modified oligonucleotides were synthesized and assayed in band shift experiments with HeLa nuclear cell extracts containing over-expressed TRs. In a first series of experiments the properties of a perfect palindrome (TRE pal) are being compared with those of a half-site and with palindromic or direct repeats containing a varied number of intervening nucleotides. Our present data show that TR alpha binds a half-site weakly, whereas direct repeats were bound at least as efficiently as TRE pal. Finally, we are correlating the in vitro receptor binding properties of the different T3REs with their ability to mediate transcriptional activation of T3RE-CAT reporter gene constructs.

Molecular Approaches to the Study of Thyroid Hormone Action

L 130 HORMONE-INDEPENDENT GENE ACTIVATION IN YEAST BY THE RAT C-*ERB A β* THYROID HORMONE RECEPTOR, **Paul G. Walfish, Yong-fan Yang, Hiroyuki Ohashi,** and **Chao Lu.** Thyroid Research Laboratory, Samuel Lunenfeld Research Institute of Mount Sinai Hospital, and Department of Medicine, University of Toronto, Toronto, Ontario, Canada M5G 1X5.
In our previous report, rat thyroid hormone receptor (TR-β, c-*erbA β*) has been successfully expressed in yeast (Lu *et al.*, *BBRC* 1990, 171; 138-42). To develop a model system for studying both TR-β protein expression and its *trans*-activating properties, a yeast reporter plasmid containing the β-galactosidase gene and a synthesized 16 bp palindromic oligonucleotide of the thyroid hormone response element as described by Glass *et al.* (1988) was co-transfected with a TR-β expression vector encoding a full-length TR-β. In agreement with the hormone binding data, TR-β (without $CuSO_4$ induction) produced a strong basal transcriptional response in the absence of added L-triiodothyronine (T3), which was 15-fold higher than that in the control (m±SE = 2448±181 *vs.* 163±13 Miller units/mg protein, respectively). Maximal transcriptional activity was observed at 4-5 hours after yeast culture enrichment. 1 μM T3 resulted in only an additional 25-30% increase in the transcriptional activity. Although constitutive stimulatory transcriptional activity was observed in a mammalian system after deletion in the putative hormone binding domain (O'Donnell & Koenig, 1990), a similar site deletion of TR-β (Δ181-380,aa; *Nhe I* deletion in rc-*erbA β* cDNA) eliminated transcriptional activity in our yeast system with or without T3. **Conclusion:** These observations in yeast are at variance with mammalian transcriptional systems which are predominantly hormone-dependent. The discordance in deleted TR-β transcriptional activities between yeast and mammalian systems also suggests that TR *trans*-activating activity may be regulated by transcriptional-system specific factor(s).

L 131 EFFECT OF MUTATIONS OF THE rGH T3 RESPONSE ELEMENT ON THE BINDING OF PURE T3 RECEPTOR α, Graham R. Williams, John W. Harney, *Barry M Forman, *Herbert H. Samuels, P. Reed Larsen and Gregory A. Brent, Thyroid Division and Howard Hughes Medical Institute, Brigham and Women's Hospital, Boston, MA 02115, and *Departments of Pharmacology and Medicine, New York University School of Medicine, New York, NY 10016.
The rat growth hormone (rGH) promoter T3 response element (TRE) is located -189 to -165 upstream of the mRNA start site. The TRE consists of 3 binding half-sites (A, B and C) with a consensus sequence AGGT(C/A)A. The wild type rGH TRE and a series of point mutations, were inserted upstream of a truncated rGH promoter and transfected into GH4C1 rat pituitary tumor cells. The wild type rGH was induced 4 fold by T3. Point mutation in each of the 3 domains A, B or C, reduced T3 induction to 2.7, 1.9 and 2.3 fold respectively. As previously reported, B and C domain up-mutations increased T3 response up to 43 fold and point mutation of the A, B or C domains, in the context of the C domain up-mutant, reduced T3 induction. Chicken T3Rα was overexpressed in E. coli, purified, and its binding to each TRE was analysed in gel shift assays. Monomer and dimer bands, as have been reported for half-site palindrome sequences (Forman et al, 1990, Abstract #1201, Endocrine Society Annual Meeting, Atlanta), were observed with the wild type rGH TRE. Up-mutants produced a more intense dimer band as well as a third band at a higher position, which we propose represents occupancy of all 3 half-sites. Down-mutants had reduced T3R affinity and, in some cases, loss of specific bands. We conclude that all 3 half-sites previously shown to be essential for full T3 induction are necessary for maximal T3R binding and that the magnitude of induction by T3 correlates directly with T3R binding affinity and pattern.

L 132 THE MOUSE PITUITARY-SPECIFIC THYROID HORMONE RECEPTOR β2 ISOFORM FROM THYROTROPIC TUMOR CELLS DIFFERS AT THE AMINO TERMINUS WHEN COMPARED TO THAT FROM RAT PITUITARY TUMOR CELLS. William M. Wood, Kenneth W. Ocran, David F. Gordon, Virginia D. Sarapura and E. Chester Ridgway. Department of Medicine, University of Colorado Health Sciences Center, Denver, CO 80262
One of the principle target organs for thyroid hormone (T_3) is the anterior pituitary gland where it acts on thyrotropes to suppress transcription from the α and TSHβ subunit genes. To begin to examine which thyroid hormone receptor (TR) isoforms are present in thyrotrope cells, we constructed a random primed cDNA library from poly A+ RNA from TtT-97 thyrotropic tumor cells which express and down-regulate both subunit genes of TSH with T_3. The library was screened with specific probes encoding the rat TRα and β isoforms. Several overlapping clones corresponding to the TRα1 and TRα2 isoforms were isolated and their nucleotide sequence was identical to mouse TRα cDNA clones previously reported from skeletal muscle and testis. Partial TRβ1 cDNAs revealed extremely high sequence conservation with the corresponding rat species with only 3 amino acid differences all located in the N-terminal region. A cDNA for the pituitary-specific TRβ2 isoform was generated using PCR and sequence information from the published rat sequence. The mouse TRβ2 sequence was quite divergent from the rat species from GH_3 pituitary tumor cells. The amino terminal β2 specific domain was 90% homologous at the nucleotide level resulting in 12 amino acid differences. However an in-frame stop codon resulted in the mouse TRβ2 protein being 39 amino acids shorter than the rat protein. Northern blot analysis revealed that TtT-97 tumors contained both TRα1 and α2 mRNAs in a ratio (α2 >> α1) similar to that in adult mouse brain. T_3 treatment resulted in a reduction in the tumor levels of the α2 mRNA but not α1 transcripts. mRNA encoding Rev-erb, the antisense transcript to α2, was also expressed. TRβ1 and TRβ2 mRNAs were also present in thyrotropic tissue but only TRβ1 signals were detected in other tissues. The tumor levels of TRβ2 mRNA but not TRβ1 were reduced by T_3 treatment.

Late Abstract

INTACT CODING REGIONS OF ALPHA 1- AND BETA 1 - THYROID HORMONE RECEPTOR GENES IN GENERALIZED THYROID HORMONE RESISTANCE

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In a family, the mother and the only children, son and daughter, were affected with generalized thyroid hormone resistance (GTHR). Diffuse goiter was a common symptom. Despite of clinical euthyroidism all three exhibited extremely elevated levels of serum T4 and T3 inclusive the free hormone fractions as well as elevated basal and TRH stimulated TSH. To investigate the pathology of this disease on the molecular level we first concentrated on possible defects in thyroid hormone receptors. Total cellular RNA from the patients' blood lymphocytes or cultured skin fibroblasts was isolated and reverse transcribed into "first strand"- cDNA using random hexamers as primer. The cDNA served as template for the amplification of the whole coding regions by the polymerase chain reaction (PCR). PCR primers were specific for part of the 5'- and 3'- noncoding regions of both receptor genes. PCR products were sequenced by the dideoxy method according to Sanger. Sequence analysis data of alpha 1- and beta 1-T3 receptor from all three patients didn't show any abnormalities relative to their deduced aminoacid sequence. For the first time these results demonstrate normal coding regions of alpha 1- and beta 1 - receptor in GTHR. In consequence, further studies should focus on other factors responsible for an altered gene expression.